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PROCEEDINGS OF THE AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS.

ELEVENTH ANNUAL MEETING.

New York, N. Y., December 28–30, 1916.

THE DIASTATIC ACTIVITY OF THE BLOOD IN DIABETES.

By VICTOR C. MYERS and JOHN A. KILLIAN.

(From the Laboratory of Pathological Chemistry, New York Post-Graduate Medical School and Hospital, New York.)

Although it has been recognized since the time of Magendie that blood possesses active diastatic properties, no studies of a conclusive nature appear to have been published relative to the activity of the blood diastase in human subjects suffering from diabetes. This has probably been due to the lack of a sufficiently delicate and accurate method.

It has been found that the procedure introduced by Lewis and Benedict[1] for the estimation of the sugar of the blood, may be excellently utilized in the estimation of its diastatic activity. Two 2 cc. samples of oxalated blood are taken, one being employed as a control. The control tube is made up to 10 cc. with distilled water, and the tube to be employed for the test to 9 cc. Both tubes are now placed in a water bath at 40°C. As soon as the contents of the tubes have been brought to this temperature, 1 cc. of 1 per cent soluble starch is added to the second tube, the contents are mixed, and incubation is then carried out for exactly 15 minutes at 40°C.

After the incubation has been completed, about 1.0 gm. of dry picric acid is at once added to each tube and the mixtures are stirred. When the proteins are precipitated, the tubes are centrifuged and the supernatant fluid is filtered. The sugar in 3 cc. portions of the filtrates is now estimated according to the technique described by Myers and Bailey.[2] Correction is made for the sugar originally present in the blood (with the aid of the control) and for the slight reducing action of the soluble starch. The results have been recorded in terms of the percentage of the soluble starch (10 mg.) transformed to reducing sugars (calculated as glucose) by the 2 cc. of blood employed.

[1] Lewis, R. C., and Benedict, S. R., *J. Biol. Chem.*, 1915, xx, 61.
[2] Myers, V. C., and Bailey, C. V., *J. Biol. Chem.*, 1916, xxiv, 147.

It is believed that 10 mg. of starch furnish a sufficient substrate for all ordinary conditions with human blood (except possibly in very severe diabetes), since practically identical results have been obtained with amounts of starch up to 40 mg. Glycogen offers no advantage over soluble starch, since the results are essentially identical. The possible error of glycolysis would appear to be a negligible one during a 15 minute period of incubation.

The diastatic activity of the blood, with this method, has been found to vary from 15 to 25 in a variety of miscellaneous conditions in the human subject. In cases of advanced nephritis with nitrogen retention and hyperglycemia, figures from 30 to 40 have been observed, while in eleven cases of diabetes the values ranged from 40 to 74. These results would suggest that the increased diastatic activity in both nephritis and diabetes (as shown by the analyses of the blood) may be the important factor in the production of the hyperglycemia in these conditions.

THE BLOOD LIPOIDS IN NEPHRITIS.

By W. R. BLOOR.

Lipemia has occasionally been reported in nephritis, and because of its importance in the study of fat metabolism an examination of the blood lipoids in a series of cases of severe nephritis was undertaken. The results showed mainly marked increases in the total fatty acids (due to fat) in the blood plasma and of lecithin in the corpuscles, with less marked and less frequent increases of fat and of cholesterol in the corpuscles. These abnormalities are essentially those found in the blood in alimentary lipemia and their presence in nephritis is believed to be due to a retarded fat assimilation which is one manifestation of a general phenomenon produced by the acidosis present in this condition.

THE LIPOIDS OF THE BLOOD IN RENAL CONDITIONS, WITH SPECIAL REFERENCE TO THE CHOLESTEROL CONTENT.

By ALBERT A. EPSTEIN and MARCUS A. ROTHSCHILD.

By means of the newly developed methods for the examination of fats and lipoids in the blood, it has been possible for us to examine nearly 100 cases of renal disease and allied conditions.

The results may be briefly summarized as follows: In chronic parenchymatous nephritis (nephrosis), particularly in the edematous stage, the lipoids of the blood are exceedingly high; the cholesterol content, to which special attention has been given in this study, has been observed as high as 1.226 gm. per 100 cc. In other renal conditions, including surgical cases, no such increase has been encountered. Occasionally a moderate rise has been found, but as a rule this was associated with some other superimposed condition.

In uremic cases, particularly those showing high nitrogen retention in the blood, the lipoid content is very much diminished. The cholesterol in such cases may fall as low as 0.080 gm. per 100 cc. Several effusion fluids from cases of parenchymatous nephritis have been examined and only traces of lipoid have been found in them. The urines of these cases, when rich in protein material, show the presence of small amounts of lipoid.

The origin of the increase of the lipoid content of the blood in the parenchymatous form of nephritis is not clear. Undoubtedly a large portion, perhaps the greatest, is due to ingested lipoid material. A certain part may be ascribed to mobilization of body fat, particularly as these cases when recuperating show extreme emaciation. This high grade form of malnutrition is undoubtedly associated with considerable tissue degeneration, so that a certain portion of the lipoid in the blood must be attributed to that source. At any rate, the finding undoubtedly represents a condition of fat non-utilization, comparable to that found in diabetes and other nutritional disorders. This is evidenced also by the fact that when a diet consisting of large amounts of protein, a small amount of carbohydrate, and practically no fat, is administered for a long period of time, the excessive lipemia and lipoidemia gradually subside.

OBSERVATIONS ON ACID-BASE EQUILIBRIUM IN THE BODY.

By JOHN HOWLAND and W. McKIM MARRIOTT.

(From the Harriet Lane Home and Department of Pediatrics, Johns Hopkins University, Baltimore.)

Known amounts of hydrochloric acid, of acid sodium phosphate, and of sodium phosphate of blood reaction were administered to normal adults. Determinations on the urine showed

that hydrochloric acid administration led to an increase in titratable acid (A) and a proportionate increase in ammonia excretion, the ratio A:NH_3 remaining constant. Acid sodium phosphate increased the titratable acid excretion but had no effect on ammonia excretion. The ratio A:NH_3 was greatly increased. Administration of phosphate mixtures of blood reaction ($P_H = 7.4$) led to a slight increase in the titratable acid and to a distinct diminution in ammonia excretion. The A:NH_3 ratio was considerably above normal limits.

These findings may be taken to explain, in part, at least, the low excretion of ammonia in the acidosis of nephritis, it having been previously demonstrated that this condition is accompanied by a retention of acid phosphate.

ADENINE AND GUANINE IN COWS' MILK.

By CARL VOEGTLIN and C. P. SHERWIN.

(*From the Hygienic Laboratory, United States Public Health Service, Washington.*)

From 100 liters of a mixed sample of cows' milk it was possible to isolate 500 mg. of adenine and 100 mg. of guanine by means of silver precipitation of the protein-free milk residue. These findings therefore prove the existence of two hitherto unknown constituents of milk.

THE ANABOLIC ACTION OF THE THYROID GLAND.

By N. W. JANNEY.

(*From the Chemical Laboratory of the Montefiore Home and Hospital, New York.*)

The catabolic action of the thyroid, as exemplified in the treatment of obesity, takes place when large doses of thyroid preparations are administered. It was studied by us in nitrogen partition experiments made on fasting dogs. A greatly increased excretion of all the nitrogenous urinary constituents, except creatinine, took place, indicating a stimulation of catabolic cellular metabolism. Fever and other toxic symptoms were likewise present. The anabolic action of the thyroid has remained obscure, as it can be demonstrated only by giving very

small doses of the gland or its preparations. The results in many respects are the opposite of the catabolic and toxic action.

Using the nitrogen balance as a gauge of the effect of thyroid treatment, we have studied the results of administering small doses of thyroid preparations to cretins and exophthalmic goiter patients over extended periods. It could be shown that an added retention of nitrogen accompanied improvement in the clinical symptoms.

The same amounts of thyroid preparations fed to normal control persons on the same diet and under the same conditions were followed either by no change or by a reduction in the nitrogen balance.

In other preliminary experiments evidence has been obtained that the anabolic action which the thyroid gland exerts on protein metabolism is connected with its effect on carbohydrate metabolism. Thus the protein-sparing action of sugar which was found to be greatly reduced after thyroidectomy could be restored by feeding in addition small doses of thyroid.

ENZYME AND SUBSTRATE IN AUTOLYSIS.

By MAX MORSE.

The following reasons may be given for believing that the effect of acid introduced or already present in an autolyzing digest is upon the enzyme, and that the effect upon substrate, discovered by Bradley, is of secondary and not primary importance.

1. All known enzymes respond to alterations in hydrogen ion concentration. Therefore, if the effect of acid is upon the substrate from the inception of autolysis, as Bradley believes, no provision is made for this response and one must conclude that the enzyme is refractory to modification of the concentration of hydrogen ions. In this case the enzyme differs from all known ferments.

2. The substrate theory demands a high degree of specificity on the part of the enzyme, owing to the belief that the amount of substrate available for the enzyme is conditioned by the concentration of hydrogen ions. At the same time, Bradley has shown that the enzyme is not specific, since foreign proteins (casein, coagulated proteins, etc.) are digested.

3. The addition of coagulated proteins to a digest together with the addition of acid does not lead to acceleration of autolysis. This is interpreted on the substrate theory by assuming that there is no further activation of the enzyme. It is equally well interpreted as meaning that no more enzyme is introduced, for on introducing enzyme the rate is increased.

4. Inorganic colloids accelerate autolysis (Ascoli and Izar). Pincussohn, Wohlgemuth, and others have demonstrated an effect upon salivary amylase, pepsin, etc., when subjected to various inorganic suspensoids, so it is probable that a similar effect is exerted upon the autolytic enzyme.

5. Calcium salts, such as phosphate, sulfate, etc., show no acceleration in autolysis when used in hydrogen equivalent solutions, while similar salts of potassium, sodium, etc., show marked acceleration. The "salt effect" of Loeb is doubtless operative here and we have a basis for the suggestion herein made that the effect of acid is first to modify the permeability of the membranes and secondly to activate the enzyme.

ANTAGONISTIC ELECTROLYTES AND JELLY FORMATION.

By G. H. A. CLOWES.

When NaCl is added in increasing proportion to aqueous suspensions of soaps or lipoids, the dispersion of the particles in water is at first promoted, reaches an optimum at approximately 0.15 M NaCl, which corresponds approximately with the concentration of this substance in the blood, as noted by Koch. Subsequently an aggregating effect is exerted, precipitation occurring at a concentration of 0.35 to 0.4 M NaCl, which appears to be the limiting concentration for the maintenance of life of marine organisms, of mice on intravenous injection, and the precipitation point for emulsions, jellies, and a variety of other physical systems.

Koch's curve of the amount of $CaCl_2$ required to precipitate lecithin at different concentrations of NaCl corresponds closely with curves obtained by the drop method[3] when NaCl is added in increasing proportion to an aqueous phase containing a constant amount of soap passed through neutral oil. The initial

[3] Clowes, G. H. A., *J. Physic. Chem.*, 1916, xx, 407.

rise in drop number attributable to preferential adsorption of Cl ions promoting dispersion of the soap particles reaches an optimum at 0.15 to 0.25 M and subsequently diminishes, presumably owing to the relatively increased adsorption of the Na ion with rise in concentration. Soap jellies may be obtained at concentrations between 0.25 and 0.4 M NaCl provided sufficient NaOH was added to the original system to insure a sufficiently high initial dispersion of the soap particles (as indicated by the optimum drop number), to prevent aggregation of the particles and their deposition under the influence of gravity. If Brownian movement is maintained up to a certain concentration of NaCl, subsequent adsorption of Na ions causing reduction of negative charge and change of surface tension results in such a distortion of the individual particles that they coalesce simultaneously with one another to form a jelly-like structure containing water in the interstices.

The close similarity in the antagonistic curves of $CaCl_2$ and NaCl and other salts having a more readily adsorbed anion in such widely diversified systems as living protoplasm, the blood clot emulsions, soap jellies and films, and soap and lipoid suspensions, and the common limiting concentrations at which individual salts like NaCl and $CaCl_2$ produce inhibiting effects in all these systems, concentrations which differ entirely from those at which similar effects may be observed on proteins, suggest the probability that protoplasmic equilibrium and the formation of reversible protoplasmic jellies are dependent upon what may be designated as an imperfect reversal of phase relations promoted by the action of antagonistic electrolytes on interfacial films of soap and lipoid just as in reversal of equilibrium in the case of emulsions of oil and water.

ELECTROLYTES AND ANAPHYLAXIS.

By G. H. A. CLOWES.

Ca salts appear to counteract sensitization phenomena by diminishing the permeability of the tissues to water. The fact that Ca salts, owing to their aggregating effect on soap films, may be used to counteract increased permeability resulting from adsorption or interaction of negative ions, and that the ratios in

which various salts having readily adsorbed anions are antagonized by salts of Ca are approximately the same in such widely diversified systems as marine organisms, living mice, blood coagulation, complement hemolysis, soap and lipoid suspensions, and drop systems,[3] coupled with the drop in blood coagulation in anaphylaxis, suggests the probability that the antigen-antibody combination affords a link between water and some non-aqueous phase in the protoplasmic film whereby the latter is subjected to the action of readily adsorbed anions already present in the system with a resulting sudden increase in permeability beyond those limits within which normal protoplasmic function may be maintained.

Support for this contention may be found in the well known surface tension effects and the rôle played by electrolytes in immune phenomena; and a practical analogy is to be found in experiments carried out by means of the drop method, demonstrating that water containing NaOH or NaOH + NaCl may be passed through neutral oil without causing any change in the drop number, and water or water + NaCl may be passed through oil containing fatty acid without change, but when NaOH and fatty acid are both present in the system either as soap in the water phase or as NaOH in the water phase and fatty acid in the oil phase, a considerable rise in the drop number occurs, and if NaCl is present there is in addition a still further rise.

With a drop system in which the conditions of equilibrium appear to be comparable with those of living protoplasms, a rise from ten to fifty drops may be effected by introducing a given amount of soap into the water phase or NaOH into the water phase and fatty acid into the oil phase, and a further rise to 300 drops may be effected by using physiologically normal salt solution instead of water. The great increase in permeability indicated, far in excess of that required to cause destruction of protoplasm, could only be effected in the presence of both alkali and fatty acid, presumably owing to the affinity of water for NaOH, of NaOH for fatty acid, and fatty acid for oil; and the consequent establishment of chemical or physical contact between the water and oil phases without which the dispersing effect of the readily adsorbed Cl ion could not come into play.

CALCIUM AND PHOSPHORUS IN THE BLOOD OF LACTATING COWS.

By EDWARD B. MEIGS and N. R. BLATHERWICK.

(From the Bureau of Animal Industry, United States Department of Agriculture, Washington.)

Calcium, nitrogen, total phosphorus, and lipoid and inorganic phosphorus have been determined in the blood and plasma of cows and heifers of various ages, on various rations, and at various stages of pregnancy and lactation. The results are still highly incomplete, but are sufficient to indicate certain relations.

The calcium content of cows' plasma has been found to be surprisingly constant. It is somewhat over 0.011 per cent in very young heifer calves, and falls off gradually with increasing age until it reaches about 0.010 per cent toward the end of the first year, from which point it remains nearly constant. Our results indicate that it is not appreciably altered by pregnancy, lactation, or by considerable changes in diet. Our highest figure for plasma calcium has been 0.0114 per cent, and our lowest 0.0086 per cent.

The total, lipoid, and inorganic phosphorus contents of normal plasma are all extremely variable. In our experiments the total phosphorus has varied from 0.0085 per cent to 0.0178 per cent; the lipoid phosphorus, from a little under 0.002 per cent to a little over 0.008 per cent; and the inorganic phosphorus, from about 0.004 per cent to about 0.008 per cent. The lipoid phosphorus has been found lowest in very young calves, highest in cows at about the 6th month of lactation, and intermediate in farrow heifers from 1 to 2 years old, and in pregnant cows at the end of the previous lactation period. The inorganic phosphorus has been found highest in young calves, and is likely to be low in mature cows just after calving. It seems to depend to some extent on the amount of grain fed.

In numerous samples of plasma, the total, lipoid, and inorganic phosphorus contents have been independently determined, and it has always been found that the sum of the lipoid and inorganic fractions is equal, within the limits of experimental error, to the total.

Our nitrogen results indicate an increase in the ratio, protein:

water, in plasma with increasing age, and some individual variation in this ratio; but little change as the result of pregnancy, lactation, or change in rations.

STUDIES IN THE MECHANISM OF ABSORPTION FROM THE COLON

By SAMUEL GOLDSCHMIDT and A. B. DAYTON.

(From the Hunterian Laboratory of Experimental Pathology, Johns Hopkins Medical School, Baltimore.)

Sodium chloride solutions of concentrations above 1.2 per cent when placed into the colon of the dog cause a passage of water into the colon contents. At the same time chlorides are passing into the blood. There is an immediate drop in the concentration of chlorides. The Δ of the colon contents shows an immediate decrease of osmotic pressure. The concentration of chlorides and the Δ at the point where the volume ceases to increase is above the concentration of chlorides and the Δ of the blood.

The concentration of chlorides finally comes to an equilibrium with the chlorides of the blood. An increase of chlorides in the blood causes a readjustment of this equilibrium.

The Δ of the colon contents is not accounted for by the chlorides; hence "other substances" have diffused into the colon from the blood.

Chlorides may be withdrawn from the blood until the concentration in the colon reaches 0.12 to 0.17 per cent, when there is a reabsorption of the chlorides; nevertheless the concentration of chlorides in the colon increases to blood level. The colon is therefore not an "irreciprocal membrane." It is essentially a permeable membrane towards sodium chloride solutions.

A MODIFICATION OF FOLIN'S METHOD FOR THE ESTIMATION OF CREATININE IN BLOOD.*

By THEODORE KUTTNER.

(From Mt. Sinai Hospital, New York.)

The modified method suggested reduces the disturbing factor of the yellow color of the picric acid, because of the smaller amount of this substance used than in the original Folin method.

*Read by invitation.

For similar amounts of blood Folin's method requires thirty-three times as much picric acid as in the new procedure which depends upon removal of the proteins before the addition of the picric acid. Details of the procedure will be published shortly.

A QUICK TITRATION METHOD FOR DETERMINING SMALL AMOUNTS OF URIC ACID.

By J. LUCIEN MORRIS.

(*From the Laboratory of Biological Chemistry, Washington University, St. Louis.*)

Precipitation of uric acid as zinc urate is used as the basis of separation of uric acid from other substances in body fluids which would interfere with a subsequent permanganate oxidation. The method is described in detail for urine but has application as well to blood. Albumin is removed by treatment either with trichloroacetic acid or *m*-phosphoric acid. An aliquot part of albumin-free filtrate is added to a special solution of sodium lactate, sodium acetate, and acetic acid, and the phosphates and ammonia are removed by precipitation at room temperature with uranium acetate. The solution obtained after filtering or centrifuging will allow its uric acid content to be removed completely as zinc urate upon the successive addition of zinc acetate and sodium carbonate. Centrifuging for a minute separates the precipitate and the liquid may be poured off. Solution of the precipitate is obtained by the addition of acetic acid and a reprecipitation with sodium carbonate assures the absence of substances which might interfere with the permanganate titration. Solution of the precipitate in acetic acid, heating to 80°C., and adding phosphoric acid until zinc phosphate dissolves are the conditions which immediately precede the titration with 0.005 N potassium permanganate. Use of sulfuric acid gives a figure about 10 per cent higher, due probably to purines which are precipitated with zinc but which are not oxidized with permanganate in acetic acid solution.

NOTE ON A TITRATION METHOD FOR DETERMINING MINUTE QUANTITIES OF ACETONE.*

By ROGER S. HUBBARD.

(*From Washington University, St. Louis.*)

The solution of iodine used in the regular Messinger determination of acetone is diluted in 3 per cent potassium iodide, and used for the determination of minute quantities of acetone. 15 gm. of potassium iodide are dissolved in water in a 500 cc. volumetric flask, appropriate amounts of the stock iodine solution added, and made to volume. From the stock solution of approximately 0.1 N concentration (1 cc. equals 1 mg. of acetone) 0.01 N and 0.002 N solutions are so prepared. Thiosulfate solutions of corresponding strength are easily prepared, and are fairly stable.

In carrying out the determination the acetone solution is distilled to a volume of 250 cc. and 50 cc. are taken for a determination. 40 cc. of 0.01 N iodine solution are added, followed by 1 cc. of 10 × N NaOH. The solution is shaken, and allowed to stand 10 minutes. It is then acidified with H_2SO_4, and the excess of iodine titrated with 0.01 N thiosulfate solution, using 1 to 2 cc. of 0.25 per cent starch as indicator.

If the amount of acetone is less than 0.2 mg., the procedure is repeated, using 10 or 20 cc. of 0.002 N iodine solution, and titrating with 0.001 N thiosulfate. A blank should be run on the NaOH used. The iodine solution is permanent for 4 or 5 days, and thiosulfate solution for 48 hours. Amounts of acetone as small as 0.01 mg. may be accurately determined by this method

THE ALLEGED NINHYDRIN REACTION WITH GLYCEROL, ETC.

By VICTOR JOHN HARDING.

(*From the Biochemical Laboratory, McGill University, Montreal.*)

The author found that in following the work of Halle, Loewenstein, and Přibřam on the production of a ninhydrin reaction by glycerol, specimens of glycerol of different origin gave different results. The production of a positive ninhydrin reaction with glycerol is believed to be due to the presence of nitrogenous impurities.

*Read by invitation.

THE USE OF HEXAMETHYLENETETRAMINE AS A RENAL FUNCTIONAL TEST, AND ITS COMPARISON WITH OTHER FUNCTIONAL TESTS.*

By W. G. LYLE.

(*From the Harriman Research Laboratory, Roosevelt Hospital, New York.*)

A method for the quantitative determination of urotropin in the urine has been described by Falk and Sugiura.[4] They found that if 15 grains of urotropin were given to a person with normal kidney function, and the urine was collected for 12 hours, they were able to recover from 40 to 80 per cent of the urotropin unchanged; on the other hand, if the patient was suffering from nephritis, little or no urotropin was excreted. It was suggested that this fact should be utilized as a test for the functional activity of the kidney. A comparison of this method with other functional kidney tests was undertaken.

In general it may be stated that in cases where the clinical symptoms point undoubtedly to nephritis nearly all the functional tests agree fairly well. In early cases, however, where the diagnosis is in doubt, urotropin is of value. The failure of the kidney to excrete the urotropin molecule is a very early symptom of interference with its function. It is of interest to note that this phenomenon coincides closely with the finding of an increased amount of uric acid in the blood, and with the changes in the kidney function, as shown by the Mosenthal test meal, especially those cases showing a marked increase in the volume of night urine. In this type of case we have frequently found a good, or even high dye test and a normal non-protein nitrogen and urea. Whether some of these cases will develop a true nephritis remains to be seen, but that there is present some disturbance of kidney function is evident.

In conclusion, from our work with these tests, one fact stands out clearly,—that it is unwise to base a diagnosis on the result of one or two tests as there are cases in which the uric acid in the blood is normal while the urotropin, Mosenthal meal, and the dye test show kidney disturbance. On the other hand, cases of gout may show a high uric acid content in the blood, while the

*Read by invitation.

[4] Falk, K. G., and Sugiura, K., *J. Pharm. and Exp. Ther.*, 1917, ix, 241.

other tests are normal. The advantage of urotropin is that the test is easily carried out and gives the patient no inconvenience. We therefore recommend that in a suspected case the patient should be given 15 grains of urotropin, dissolved in a glass of water, shortly before the evening meal, at which time fluid should be taken to the amount equivalent to two more glasses of water; a 12 hour specimen should be collected and examined for urotropin; if none is found, or a small amount, the other tests should be carried out.

THE CHOLESTEROL CONTENT OF THE BLOOD IN VARIOUS HEPATIC CONDITIONS.*

By M. A. ROTHSCHILD and J. FELSEN.

(From the Department of Physiological Chemistry, Pathological Laboratory, Mount Sinai Hospital, New York.)

In obstructive icterus the cholesterol content of the blood is markedly elevated and bears a definite relationship to the intensity of the icterus. In conditions such as cirrhosis, acute yellow atrophy, pernicious vomiting of pregnancy, or extensive carcinomas of the liver, the cholesterol content of the blood is not increased and is usually reduced. The cholesterolemia is thus not proportionate to the intensity of the icterus. In other forms of icterus, as seen in Banti's disease, Gaucher's disease, splenomegaly with icterus, and pernicious anemia and other blood diseases, there is no increase in the blood cholesterol, and in most of these conditions the amount present is decidedly less than normal.

THE USE OF PANCREATIC VITAMINE IN CASES OF MALNUTRITION.

By WALTER H. EDDY.

(From the Department of Pathology of the New York Hospital.)

The report covers the preliminary study of the use of pancreatic vitamine in cases of marasmus. Up to the present time the treatment has been administered to eleven cases, seven of which form the basis of the present paper; the other four are not sufficiently advanced to supply data at this time.

* Read by invitation.

The treatment consists in feeding children 2 gm. per day of Lloyd's reagent which has been activated by pancreatic vitamine after the method of Seidell. Previous work had demonstrated that 50 gm. of Lloyd's reagent shaken with 1 liter of the water-soluble portion of an alcohol extract of pancreas remove from that volume its growth-stimulating power. In these experiments 1 gm. of Lloyd powder contains the vitamine from 54 gm. of pancreas. (This assumes that the removal of the vitamine is quantitative.) The powder is administered by mixing 1 gm. with a feeding of cereal. No difficulties have arisen in getting the children to take this material. The success of the experiment is judged by noting whether growth develops after administration of the vitamine, no other change being made in the diet.

The results suggest at least the possibility of stimulating assimilation by the artificial feeding of pancreatic vitamine. The dosage and the conditions favorable and unfavorable to its effectiveness are under investigation.

THE URIC ACID CONTENT OF THE BLOOD OF THE NEW-BORN.

By F. B. KINGSBURY, J. P. SEDGWICK, and L. J. ROBERTS.

Uric acid determinations in the blood of the new-born showed that the concentration of uric acid varied from 1.8 to 3.5 mg. per 100 gm. of placental blood, and from 2.7 to 4.9 mg. per 100 gm. of blood drawn from the new-born 8 to 120 hours later. The uric acid content in the case of a new-born 8 hours old was 4.9 mg. per 100 gm. of blood. At the end of 10 days this value had dropped to 1.4 mg. In the case of another baby the placental blood contained 1.9 mg. which rose to 4.0 mg. 46 hours later, and at the end of 9 days was 3.8 mg. Both placental blood and blood taken a few hours after birth contain more uric acid than that of the normal adult. A definite increase after birth is shown and this occurs during the same period of time that uric acid infarcts in the kidney are known to be formed.

THE PARTITION OF NON-PROTEIN NITROGEN IN THE BLOOD OF FRESH WATER FISH.

By D. WRIGHT WILSON and EDWARD F. ADOLPH.

(From the Laboratory of Physiological Chemistry, Johns Hopkins University, Baltimore, and the United States Fisheries Biological Station, Fairport, Iowa.)

The whole blood and plasma from several species of fresh water fish were analyzed for non-protein nitrogen, urea, ammonia, amino nitrogen, uric acid, creatinine, and creatine. The fish studied were the sturgeon, gar, carp, croppie, catfish, and sheepshead. The total non-protein N was lower in the ganoids (29 to 31 mg. per 100) than in the teleosts (42 to 59 mg.). The plasma contained less than the whole blood in all cases. The urea nitrogen was unusually low in most specimens, varying from 1.1 to 16 mg. per 100 cc. of whole blood and from 0.4 to 13 mg. per 100 cc. of plasma. The urea content of the plasma was less than whole blood in all cases. The amino nitrogen fraction made up from 60 to 81 per cent of the total non-protein nitrogen of the whole blood, and from 33 to 45 per cent of the total non-protein nitrogen of the plasma. The ammonia, uric acid, and creatinine contents of whole blood were similar to the other values reported for fish. The creatine content of plasma was higher than that of the corpuscles. The considerable variations in the composition of the blood of fish and higher animals suggest differences in metabolism.

THE DISTRIBUTION OF CREATININE AND CREATINE BETWEEN THE CORPUSCLES AND PLASMA OF THE BLOOD.

By ANDREW HUNTER and WALTER R. CAMPBELL.

(From the Department of Pathological Chemistry, University of Toronto.)

In forty-eight specimens, two of which came from rabbits and the remainder from human subjects, the creatinine contents of the whole blood and of the plasma were separately determined. Creatine estimations were similarly carried out in forty-two cases, in thirty-three of which the measurement of corpuscular volume enabled us to calculate the concentration of both substances in the corpuscles also. These data were supplemented in twenty-

six cases by a determination of total and preformed creatinine in the urine excreted at the time of the observation.

The single generalization which we can draw in the meantime from our results is that the concentration of both creatine and creatinine is considerably lower in plasma than in whole blood, so that, bulk for bulk, the corpuscles are much richer in these substances than the plasma. The *plasma* creatinine of normal individuals was always less than 1 mg. per 100 cc.; in all but two it fell between 0.72 and 0.92, the average being 0.82. The plasma creatine in the same subjects ranged from 0.94 to 3.5; but in two-thirds of them it lay between 1.4 and 1.7, and the average for human individuals was 1.6. The creatine and creatinine content of the *corpuscles* varied within rather wide limits, but the second was always present in much greater quantity than the first.

When creatinine is retained, as in the nephritic cases, it appears generally to accumulate in the corpuscles as well as the plasma. We have, nevertheless, encountered some cases in which the plasma creatinine seemed to have increased out of proportion to that of the whole blood, and others in which blood containing an abnormally large quantity of creatinine yielded a plasma with the normal amount. These occasional findings encourage us to hope that the plasma creatinine, as distinct from the blood creatinine, may prove to have a diagnostic value of its own.

The concentration of creatine in the plasma does not appear to bear any relation to the occurrence of creatine in the urine. We have obtained no indication of the existence of a threshold for creatine.

Observations on parturient women seem to indicate that there is a ready interchange of both substances between the mother and the fetus. The concentration of each is nearly the same in both bloods, and especially in the plasmas. Where a difference exists it is almost always in the direction of a somewhat higher concentration in the placental blood. In the majority of our cases the creatinine content of maternal and placental blood was above the normal.

The results reported are of a preliminary character only, and the work is being continued along each of the lines suggested.

THE EFFECT OF ARTIFICIAL RESPIRATION ON GLYCEMIA.

By I. S. KLEINER and S. J. MELTZER.

(*From the Department of Physiology and Pharmacology of The Rockefeller Institute for Medical Research.*)

The hyperglycemia which is produced by an intravenous injection of magnesium sulfate was shown in a previous report[5] not to be due to asphyxia, as had been suggested.[6] In those experiments artificial respiration had been given in order to prevent asphyxia, and, nevertheless, a marked hyperglycemia had been caused by the magnesium injection. It occurred to us later that possibly these results might have been due simply to the artificial respiration, since Henderson and Underhill[7] have reported that acapnia may produce an increase in the percentage of blood sugar. We have therefore determined the influence of artificial respiration upon the blood sugar concentration.

Ten dogs were used in this work. Under local anesthesia a cannula was inserted in a femoral artery and a tracheal cannula introduced for artificial respiration. In five experiments there were 20 or 21 interruptions of the air current per minute, and in five 40 to 44 per minute. Artificial respiration of the ordinary type, but with comparatively high air pressure, was administered for 1 hour. In none of the experiments was there any marked increase in the blood sugar, 0.04 per cent being the greatest increase in any. In five out of the ten experiments there was no increase whatever.

It is thus evident that vigorous artificial respiration for 1 hour causes no appreciable effect upon the percentage of sugar in the blood and therefore the hyperglycemia present when $MgSO_4$ is injected cannot be the result of an overventilation by the artificial respiration.

THE NATURE OF THE BLOOD SUGAR.

By HUGH McGUIGAN.

(*From Northwestern University Medical School, Chicago.*)

The work is based mainly on the known fact that picric acid does not interfere with the determination of blood sugar by

[5] Kleiner, I. S., and Meltzer, S. J., *J. Biol. Chem.*, 1916, xxiv, p. xx.
[6] Underhill, F. P., and Closson, O. E., *Am. J. Physiol.*, 1906, xv, 321.
[7] Henderson, Y., and Underhill, F. P., *Am. J. Physiol.*, 1911, xxviii, 275.

Fehling's solution. The results, however, were corroborated by other methods. If the filtrate from the picric acid is analyzed directly it is found to contain on the average about 25 per cent of the total blood sugar. When, however, the picric acid filtrate is boiled with 4 per cent acetic acid for 2 minutes the sugar yielded rises on the average four times. The results indicate a hydrolysis probably of a dextrin. The dialyzed serum gives the same results. Ether anesthesia changes the ratio of the polysaccharide (?) to the dextrose from 4:1 approximately to 1:1. In severe diabetes the ratio is approximately 1:1.

There is a possibility of an interfering substance in the picric acid filtrate which may hold the cuprous oxide in solution, but we have been unable to find such a body. Consequently we think that any one method of blood sugar analysis is insufficient, that the ratio of the free to the combined or polysaccharide should be determined, and that the ratio of the two is of significance in diabetes. The polysaccharide or combined form corresponds to what has been called virtual sugar.

THE ENZYMES OF THE TUBERCLE BACILLUS.

By H. J. CORPER and H. C. SWEANY.

(*From the Municipal Tuberculosis Sanitarium of the City of Chicago.*)

Tubercle bacilli both of the human and bovine variety possess autolytic enzymes, as indicated by the liberation at incubator temperature of non-coagulable nitrogen, and of amino-acid α-nitrogen after the bacilli have been killed by toluene and chloroform. The bacilli themselves, or autolysates therefrom, also possess a trypsin-like enzyme capable of splitting proteins in alkaline solution, a weak pepsin-like enzyme capable of splitting proteins in acid, an erepsin-like enzyme capable of decomposing peptone in acid solution, a nuclease capable of acting upon nucleic acid, and a urease capable of decomposing urea. The tubercle bacilli, or autolysates therefrom, do not possess enzymes capable of hydrolyzing starch or inverting saccharose at incubator temperature, at least not to an extent capable of demonstration by the delicate Lewis and Benedict colorimetric picramic acid method. Autolysates from tubercle bacilli do not possess enzymes capable of digesting elastic tissue prepared from lamb's

lung, or connective tissue prepared from tubercles, at least not by the methods used for demonstrating these enzymes. The demonstration of the latter enzymes of necessity had to be by indirect methods of attack and are, therefore, open to criticism.

A STUDY OF FOOD FISHES. THE COMPLETE ANALYSIS OF TWENTY COMMON FOOD FISHES, WITH ESPECIAL REFERENCE TO A SEASONAL VARIATION IN COMPOSITION.

By E. D. CLARK and L. H. ALMY.

(*From the Food Research Laboratory, Bureau of Chemistry, United States Department of Agriculture, Washington.*)

In the present investigation specimens of twenty different species of fish were analyzed, some only once and others twice or more times during the year. In general, there is little variation in the amount of nitrogenous and ash constituents, but fat is the great variable, its amount depending upon the season, age of fish, and relation to time of spawning and feeding. The typical migratory fish, like shad, bluefish, weakfish, and mackerel, sometimes show variations from less than 0.5 per cent to over 16 per cent, depending upon the factors mentioned above. On the other hand, typical bottom fish, like cod, haddock, and flounders, have a minimum amount of fat which varies but slightly compared with surface and migratory species. Analysis of individuals taken from the same school of fish also show variations in fat content from 1 to 8 per cent in the case of weakfish. Some species, like shad and salmon, are fattest in the spring, while others, like bluefish and butterfish, are at their best in the fall just after the active feeding period. In the case of shad, the percentage of fat decreased from a maximum of over 14 per cent in April before spawning to a minimum of less than 3 per cent in June when the fish were spent after spawning.

In the case of the nitrogenous constituents of fish there is much less variation than in the fat. The coagulable and hot-water-soluble constituents (gelatin-like substances) vary somewhat with the species but change little from spring to autumn or winter. There were striking differences in the percentages of ammonia nitrogen, but it is not certain whether they were due to individual or species differences or to the varying leaching effect of water from melting ice in which the fish were packed for shipment.

A NOTE ON CERTAIN CHEMICAL CHANGES IN SHUCKED OYSTERS UNDER REFRIGERATION, AND METHODS OF DETECTING THESE CHANGES.

By E. D. CLARK and L. H. ALMY.

(From the Food Research Laboratory, Bureau of Chemistry, United States Department of Agriculture, Washington.)

The work reported deals with chemical methods of supplementing bacterial counts in the analysis of oysters. Preliminary experiments showed that freshly shucked oysters standing at temperatures above the freezing point yielded regularly increasing percentages of ammoniacal nitrogen by aeration, amino-acid nitrogen, and reducing substances.

Larger scale studies were then made upon lots of oysters held at ice box temperatures (45° F.) and in cold rooms at 40° F. and 34° F., respectively. All these temperatures are regularly employed in the commercial handling and transportation of oysters, either in car lots or small packages. To simulate commercial practices in handling and transporting oysters, samples were held at different temperatures for various periods of time up to 15 days. During storage the ammoniacal nitrogen showed the most marked increases, but the low temperature of 34° F. had a great inhibiting effect on the production of ammonia. The amino-acid nitrogen and reducing substance (probably sugar) did not show increases of the same magnitude as ammonia because they may have been assimilated by bacteria, while ammonia is an end-product of bacterial metabolism. The amino-acid nitrogen was determined by Van Slyke's gasometric method and by Sörensen's formol titration method, both of which procedures gave satisfactory and concordant results on oyster extracts. The reducing substances were estimated by Benedict's quantitative method for sugar, using alkaline copper solution.

In general, the lower temperatures greatly retarded the formation of ammonia during storage, but apparently did not affect the other changes to as great an extent, possibly because increases in amino-acid nitrogen and reducing substances were due to enzymes which were much less retarded by low temperatures than was the growth of bacteria concerned in the production of ammonia. The reducing substance may have been glucose

arising from the hydrolysis of glycogen in the oyster tissues. Living oysters, or those suddenly killed by plunging into boiling water, contained hardly a trace of reducing substance. As is the case with all perishable substances, decomposition in oysters is not prevented by low temperatures but is merely retarded, especially at temperatures near the freezing point.

PRELIMINARY OBSERVATIONS ON THE REGULATION OF THE CALCIUM EXCRETION IN THE DOG.

By MAURICE H. GIVENS.

(From the Sheffield Laboratory of Physiological Chemistry, Yale University, New Haven.)

A dog weighing 13.3 kilos has been maintained in nitrogenous equilibrium over a period of 60 days, during which time the calcium balance has been negative. This was brought about by feeding on a meat, lard, cracker meal diet. When for the cracker meal dried skimmed milk and sucrose were substituted, on the basis of nitrogen and calorie equivalent, there was an approach toward a calcium balance. The dried skimmed milk diet contained six and a half times as much calcium as the meat, cracker meal ration.

When sodium bicarbonate (0.5 gm. per kilo) was added to the meat, cracker diet, no effect was produced on the calcium output. No effect was observed on the addition of bicarbonate to the dried milk feed.

On the other hand, when hydrochloric acid (0.11 gm. per kilo) was added to the meat, cracker diet, there was a prompt increase in the excretion of calcium by way of the kidneys, presumably through diversion from the intestinal path. When the acid was superimposed upon the high calcium diet there was likewise an increased urinary excretion of calcium though not at the expense of the fecal output.

The reported effect of alkali bicarbonate upon the calcium output during various levels of calcium intake has been confirmed on a second dog. These studies are being continued.

THE INFLUENCE OF ACID AND ALKALI ON THE ABSORPTION OF GLUCOSE FROM THE INTESTINE.

By JOHN R. MURLIN.

(From the Physiological Laboratory of Cornell University Medical College, New York City.)

Loops of the small intestine of chloretonized dogs were measured off so as to be as nearly equal in length as possible, washed out with warm Ringer's or Tyrode's solution, and then filled with equal quantities of glucose solutions, one having a certain strength of alkali measured as anhydrous sodium carbonate, and the other an equivalent amount of hydrochloric acid. The solutions were permitted to remain in the intestine for from 30 to 165 minutes, then withdrawn, and titrated for sugar with Benedict's solution. Out of nearly two dozen experiments only two failed to show more absorption from the alkaline medium than from the acid, and in one of these there was sign of leakage through the ligatures.

The following may be cited as examples.

Dog No.	Glucose given.	Strength of alkali.	Absorption per minute.		
			Alkaline loop.	Acid loop.	Length of time.
	gm.		*mg.*	*mg.*	*min.*
II	2.97	0.3 per cent.	20	10	34 and 37
III	2.94	0.5 " "	20	12	120
IV	2.14	0.3 " "	13	10	165
VI	1.90	0.3 " " in both loops.	14	15	90
A	2 per cent.	0.1 N	0.36	0.63	30
B	2 " "	0.2 "	0.035*	0.166*	45

* Per cm. length. The loops were not equal in length.

In general, the greater the concentration of the acid and alkali, the greater was the difference in absorption between the two loops. In several instances the mucous lining of the alkaline loop was ground and hydrolyzed with hydrochloric acid, but no reducing substance was found.

The explanation of the difference seems to be the different effects of alkali and acid on protoplasm, the one tending to increase permeability, the other to decrease it.[8]

[8] Osterhout, W. J. V., *J. Biol. Chem.*, 1914, xix, 335.

AN EXPERIMENTAL STUDY OF LIPOLYTIC ACTIONS.

By K. GEORGE FALK.

(*From the Harriman Research Laboratory, Roosevelt Hospital, New York.*)

The inactivation of esterase and lipase from castor beans by acids, bases, neutral salts, alcohols, acetone, esters, and heat led to the hypothesis that the lipolytically active grouping is the tautomeric enol-lactim form of the peptide linking which becomes inactive on rearrangement to the keto form. Experiments testing this view resulted in the production of lipolytically active substances by the action of alkali on castor bean globulin, casein, and gelatin. Further confirming evidence was obtained on studying the ester-hydrolyzing action of glycine, glycyl-glycine, and hippuric acid at different hydrogen ion concentrations. Summarizing, it may be said that, given a definite chemical grouping the nature of which has been indicated, and which may be present in different classes of substances, certain definite lipolytic actions will result.

A METHOD FOR THE SEPARATION OF THE DIETARY ESSENTIAL, "FAT-SOLUBLE A," FROM BUTTER FAT.

By E. V. McCOLLUM, N. SIMMONDS, and H. STEENBOCK.

(*From the Laboratory of Agricultural Chemistry, University of Wisconsin, Madison.*)

Fat-free milk, when included in a diet consisting otherwise of purified food substances, promotes growth and prevents decline of animals in a manner which indicates that it still contains the fat-soluble essential in appreciable amount. This led us to suspect that it is appreciably soluble in water. Experiments have been conducted in which butter fat was employed which had been melted and thoroughly agitated with twenty successive portions of water. After this treatment it is no longer effective in inducing growth when fed with suitably constituted diets. Change to unwashed butter fat was followed by prompt resumption of growth.

ANTINEURITIC SUBSTANCES FROM EGG YOLK.

By H. STEENBOCK.

(*From the Laboratory of Agricultural Chemistry, University of Wisconsin, Madison.*)

By means of neutral solvents there was prepared a water-acetone-soluble fraction from egg yolk which in small doses by intraperitoneal injections was able to cure a pigeon suffering from polyneuritis. The antineuritic principle was found stable to concentrated hydrochloric acid at 98° and to concentrated alkalies at room temperature, but readily destroyed by dilute alkalies at the boiling temperature. Phosphotungstic acid precipitated it incompletely. It was not adenine.

THE ISOLATION OF STACHYDRIN FROM ALFALFA HAY.

By H. STEENBOCK.

(*From the Laboratory of Agricultural Chemistry, University of Wisconsin, Madison.*)

l-Stachydrin has been isolated in pure form and as the hydrochloride from the phosphotungstic acid fraction of the water-soluble constituents of alfalfa hay. For its identification the picrate, chloroplatinate, aurate, methyl ester, and methyl ester chloroaurate were prepared and found characteristic. The optical activity was found slightly below the reported value though of the same sign. Both the Kossel and the Van Slyke methods when applied directly to alfalfa hay nitrogen give erroneous values for the diamino-acids, the error in the first case falling upon the lysine fraction and in the second case upon the histidine fraction.

A METHOD FOR THE IDENTIFICATION OF PHENYLALANINE-URAMINO-ACID IN THE PRESENCE OF UREA AND AMINO-ACID.*

By ALICE ROHDE.

So far it is undetermined whether uramino-acids exist preformed in the body fluids or whether when isolated they are the products of the interaction of urea and of amino-acid present.

* Read by invitation.

Large intravenous doses of phenylalanine were administered to cats under urethane anesthesia, the urine was collected from bladder cannulas, and the urea completely destroyed as secreted by urease. After extraction of the urea-free material with acetic ether the aqueous residues left on distillation with steam were analyzed for traces of uramino-acid by the gasometric method for the determination of amino nitrogen. No phenylalanine-uramino-acid could be detected. The determination of phenylalanine-uramino-acid added to urine and Jack bean extract is approximately quantitative by this method, so that one may expect to identify minute amounts of preformed uramino-acid if present.

THE ABSORPTION OF ALCOHOL AND ITS CONCENTRATION IN THE URINE WHEN INJECTED BY RECTUM.

By T. M. CARPENTER and E. B. BABCOCK.

(*From the Carnegie Nutrition Laboratory, Boston.*)

Studies were made with four medical students of the absorption of ethyl alcohol when introduced by the rectum. Concentrations of 5, 7.5, and 10 per cent were used and amounts varying from 200 to 1,000 cc. were introduced into the rectum. The average amount of alcohol given was about 25 gm. The solutions were retained from 2 to 5 hours. The unabsorbed alcohol was determined in the washouts by the Nicloux method. The absorption of alcohol was practically complete in all cases, the highest amount unabsorbed found being about 0.5 gm.

The concentration of alcohol in the urine was also determined by collecting the urine in as short periods as possible after the alcohol was introduced into the body either by mouth or by rectum. The maximum concentration was about 0.40 mg. per 1 cc. and appeared between the 1st and 2nd hours after ingestion. It fell to zero in about 5 to 6 hours. In one subject there was a tendency for the alcohol percentage to be higher when it was taken by mouth; in the other the concentrations were about the same irrespective of the method of ingestion.

THE COMPOSITION OF RENAL AND VESICAL CALCULI.

By SAMUEL BOOKMAN.

In the quantitative analysis of fourteen stones, it is proven that the alkalinity of the urine is probably at no time sufficiently strong to dissolve the uric acid and urate stones, neither is it acid enough to cause the solution of oxalate and phosphate stones, which will account for the formation of mixed stones. The percentage composition of these stones exemplifies the change from the acid to the alkaline precipitation, or the reverse, the phosphates and carbonates being precipitated in the alkaline condition, and the urates and uric acid in the more acid reaction, the oxalates in the less acid urines, as well as in the alkaline condition. No doubt the disturbance in the colloid equilibrium plays an important part in this precipitation, but in addition we have the following contributing conditions to reckon with as well: diet, acidity, concentration, inflammation, and bacterial decomposition within the urinary tract.

THE ACTIVE CONSTITUENT OF THE THYROID: ITS ISOLATION, CHEMICAL PROPERTIES, AND PHYSIOLOGICAL ACTION.

By E. C. KENDALL.

(*From the Mayo Clinic, Rochester, Minn.*)

Primary cleavage of thyroid proteins results in acid-insoluble A and acid-soluble B constituents. Separation of the iodine compound from A is accomplished by the solubility of its barium compound in barium and sodium hydroxides. Many successive precipitations, alternated with heating in sodium hydroxide and the action of carbon dioxide, remove impurities, the last traces of which are removed by dissolving in alkaline alcohol and precipitating with acetic acid. The active constituent separates in microscopic needles. It may be precipitated as free base or in salt form. The free base is insoluble in alcohol and contains 65 per cent of iodine. The sulfuric acid salt is soluble in alcohol and contains 60 per cent of iodine. Its molecular weight appears to be 586. The chemical properties depend upon the degree of purification and the presence of other substances. In impure form heating with acid destroys the physiological activity and gives a product resembling Baumann's iodothyrin.

The physiological activity depends upon the amount administered and the susceptibility of the individual. 0.125 to 0.25 mg. daily is sufficient for cretinism. The maximal quantity tolerated by human beings so far tried has been 2 mg. daily. The response is not immediate; 24 to 72 hours may elapse before the physiological effects appear. So called hyperthyroidism symptoms and death follow administration of excessive doses to animals.

NEW BUFFER MIXTURES, STANDARD TUBES, AND COLORIMETER FOR DETERMINING THE HYDROGEN ION CONCENTRATION OF SEA WATER.*

By J. F. McCLENDON.

(From the Physiological Laboratory of the University of Minnesota, Minneapolis.)

Two stock solutions were used: (1) 0.3 M boric acid containing 2.25 per cent NaCl, and (2) 0.075 M borax containing 1.9 per cent NaCl, and mixed in thirty different proportions that may be estimated by plotting from data in the following table. The first column gives the percentage of the boric acid solution, the second that of the borax solution, the third the P_H of sea water of 13.9 gm. Cl per liter, the fourth the P_H of sea water of 17.7 gm. Cl per liter, and the fifth the P_H of sea water of 21.5 gm. Cl per liter. The tubes of borate mixtures and sea water have been calibrated fifty times by means of the hydrogen electrode, and recalibrated by the same means at Princeton University.

79.5	20.5	7.50	7.45	7.40
72	28	7.70	7.65	7.60
68	32	7.80	7.75	7.70
60	40	8.00	7.95	7.90
37	63	8.50	8.45	8.40
8	92	9.00	8.95	8.90
1	99	9.10	9.05	9.00

Thymolsulfophthalein was used as indicator from P_H 7.9 to 9 and *o*-cresolsulfophthalein from P_H 7.5 to 8.3, so as to form 0.001 per cent solution in both buffers and sea water. The buffers were sealed in "Nonsol" tubes of 24 mm. bore and the sea water

* Read by title.

was placed in open tubes of the same bore, and compared in a colorimeter having the lenses of a stereoscope placed in contact so as to bring the centers of the tubes together in a sharp line when viewed from the side with one eye 6 inches from the lenses. Tables were prepared for determining the CO_2 tension, CO_2 content, O_2 content, and heat given off in the respiration of marine animals.[9]

THE INFLUENCE OF TEMPERATURES ABOVE FREEZING ON THE CHANGES IN CHEMICAL COMPOSITION, BACTERIAL CONTENT, AND HISTOLOGICAL STRUCTURE OF THE FLESH OF THE COMMON FOWL.

By M. E. PENNINGTON, J. S. HEPBURN, E. Q. St. JOHN, and E. WITMER.

(*From the Food Research Laboratory, Bureau of Chemistry, United States Department of Agriculture, Washington.*)

Chickens were killed and picked, and were kept at a temperature of approximately 0°C. for 24 hours, in order to remove the animal heat. Some birds were then analyzed as "freshly killed chickens;" others were kept in the room (average temperature 23.9°C., maximum holding period 4 days), or in a house refrigerator (temperature 7.2–12.8°C., maximum holding period 7 days), or in a mechanically refrigerated "chill room" (temperature 0°C., maximum holding period 3 weeks), and were then analyzed. A number of analyses were made, both of freshly killed birds and of birds kept at each of the temperatures stated. While the total nitrogen content of the muscle remained unchanged, the partition of the nitrogen underwent changes at all three temperatures of holding. Thus the total nitrogen of the aqueous extract invariably increased in the chickens kept in the room, the increase being a direct function of the period of holding; in the refrigerator this fraction of the nitrogen at times either increased or decreased, depending on the period of holding; in the chill room it decreased during the first 2 weeks of holding, then increased. At all three temperatures of holding, the amino-acid and basic nitrogen increased at the expense of the protein nitrogen, usually in a progressive manner. The most striking change in the fat constants

[9] The sealed tubes and colorimeter may be obtained from Hynson, Westcott, and Dunning, Baltimore.

was an increase in the acid value, which became progressively greater as the period of holding lengthened. Both the formation of amino-acid and basic nitrogen, and the increase in the acid value of the fat occurred most rapidly at the temperature of the room, least rapidly at that of the chill room. Frequently the saponification number and the Hehner number of the fat increased or decreased *simultaneously*. The bacteria, which grow under aerobic conditions, increased in both the muscles and the skin at all three temperatures of holding; the increase was enormous in the chickens kept in the room, and was less marked, though distinct, in those kept in the refrigerator, and in the chill room; the increase usually became greater as the period of holding lengthened. Organisms which liquefy gelatin were comparatively few in number, even in the chickens which had been kept in the room, and in which the proteolysis had become most advanced. The muscle cells changed in width at all three temperatures of holding. A pronounced swelling or increase in width occurred at the temperature of the room, and a slight increase in width at the temperature of the refrigerator. A shrinkage or decrease in width of the muscle cells—usually progressive—took place at the temperature of the chill room. At all three temperatures, room, refrigerator, and chill room, rupture of the muscle fibers and exudation of the cell contents occurred; the muscle, its nerves and blood vessels, and the connective tissue became altered in histological structure and in the staining power of their various elements; these changes in structure and in affinity for stains depended in part on the temperature, and in part on the time of holding.

The changes which occurred during holding in the chill room for 3 weeks were about equal to those occurring during holding in the house refrigerator for 5 to 7 days. The changes which took place during holding in the house refrigerator for 5 days were less than those taking place during holding in the room for 2 days.

THE INFLUENCE OF TEMPERATURES BELOW FREEZING ON THE CHANGES IN CHEMICAL COMPOSITION, BACTERIAL CONTENT, AND HISTOLOGICAL STRUCTURE OF THE FLESH OF THE COMMON FOWL.

By M. E. PENNINGTON, J. S. HEPBURN, E. Q. St. JOHN, and E. WITMER.

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Chickens were killed and dry picked and kept at 0°C. for 24 hours to remove the animal heat. They were next frozen solid by holding for 48 hours in a "sharp freezer" at a temperature not higher than −14.4°C., then were stored in mechanically refrigerated "freezers" at a temperature of −9 — −13°C. for definite periods of time, the maximum period being 2 years. Every 3 or 4 months, several samples were taken from the freezer and analyzed. The "freshly killed chickens" of the preceding research were taken as the fresh standards with which to compare these hard frozen chickens. The muscular tissue showed a progressive desiccation, or loss of water, while the chickens were held in the freezer. The total nitrogen of the muscle remained unchanged; however, changes occurred in the partition of the nitrogen. Thus the total nitrogen of the aqueous extract increased or decreased, depending on the period of holding. The amino-acid and basic nitrogen showed small but progressive increases; this indicated that a slow but distinct proteolysis had occurred, leading to the accumulation of non-protein nitrogenous compounds at the expense of the protein. The acid value of the fat increased; the saponification number and the Hehner number decreased during the early months of holding, then increased. The hydrolysis of the fat and the digestion of the protein occurred far less rapidly than in chickens held at temperatures above freezing.

The bacteria, which grow under aerobic conditions, decreased in number in the muscle during the freezer storage; the decrease was apparent at the end of 4 months, became more marked as the period of holding lengthened, and was most marked after the birds had been in the freezer for 1 year or longer. Organisms which liquefy gelatin and those which grow in an anaerobic

environment were present only occasionally in the chickens from the freezer; organisms of putrefaction, which grow under anaerobic conditions after their suspension in physiologic salt solution has been kept at 80°C. for 10 minutes, were present still less frequently. The evidence points to an enzymic rather than to a bacteriologic cause for the foregoing chemical changes.

The phenomenon of freezing in itself produced little, if any, change in the width of the muscle cells, as was shown by measurement of sections cut from different pieces, removed from the same muscles before and after freezing the chickens at an average temperature of −11°C. in the "freezer." However, during prolonged holding in the freezer, a progressive shrinkage or decrease in width of the cells occurred. Muscle fibers with a ruptured sarcolemma and exuding cell contents became more numerous as the period of holding lengthened. Characteristic changes took place in the histological structure of the muscle cells, nerve fibers, blood vessels, and connective tissue, and in the affinity of their elements for stains. When chickens were stored in the freezer in closed metallic containers, desiccation was prevented; the other changes were retarded but not prevented. The changes here recorded were most marked *after* the normal commercial period of freezer storage had been exceeded. Both the analyses and organoleptic tests showed that no loss of food value had occurred while the chickens were in the freezer; the birds were still wholesome and nutritious; the only appreciable change was a loss in flavor after holding in the freezer for a period greater than 9 months.

The changes in chemical composition during holding in the freezer for 1 year were about equal to the changes which occurred during holding in the chill room for 3 weeks.

AUXO AMYLASES.

By ELBERT W. ROCKWOOD.

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Many amino-acids increase the hydrolytic cleavage of starch by ptyalin. This is true of the α-amino-acids such as glycocoll and tyrosine which result from the hydrolysis of proteins. The accelerating effect is not lost by substituting other radicals for

hydrogen of the amino group, as in hippuric acid. Where the amino group is substituted in the carboxyl the compound has no stimulating effect, as in acid amides including urea. Introduction of the sulfonic radical neutralizes the effect of the amino group, as in sulfanilic acid. The position of the amino group, as in the aminobenzoic acid isomers, does not modify their activity; all stimulate the ptyalin. Compounds of amino-acids, like the proteins, also increase the amylolytic activity of ptyalin. As far as has been determined the pancreatic amylase is similarly affected by the amino-acids. The work is being continued.

A CHEMICAL STUDY OF PROLONGED INANITION.

By R. E. SWAIN.

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The subject studied was a tailor by occupation, 30 years of age, who voluntarily subjected himself to a period of starvation which was continued for 60 days. The body weight was about 61 kilos at the beginning of the fast and about 36 kilos at the end. The excreta were collected for analysis from the 40th to the 60th days, and at intervals during this period the body temperature, pulse rate, blood counts, and other clinical data were recorded. The total urinary nitrogen output was maintained at a higher level than that recorded in previous cases of fasting, and shows a rise from the 53rd to the 56th days similar to the premortal rise in lower animals. This rise was followed by a rapid decrease in the daily excretion for the rest of the period. Ammonia is relatively very low. Creatinine was fairly constant until the 54th day when, following the rapid increase in the total nitrogen output and subsequent rapid fall, it decreases in amount to the end of the fast. Creatine is relatively high, exceeding the creatinine in amount during the period of high total nitrogen.

ADENINE-URACIL DINUCLEOTIDE AND THE STRUCTURE OF YEAST NUCLEIC ACID.*

By WALTER JONES and B. E. READ.

*Published in full in *J. Biol. Chem.*, 1917, xxix, 111.

STUDIES OF AUTOLYSIS.

V. THE INFLUENCE OF BILE ON AUTOLYSIS.*

By H. C. BRADLEY and JOSEPH TAYLOR.

THE COMPOSITION OF ADIPOCERE.**

By R. F. RUTTAN and M. J. MARSHALL.

THE USE OF COTTON SEED AS FOOD.†

By THOMAS B. OSBORNE and LAFAYETTE B. MENDEL.

CYSTINE AND THE MINIMUM PROTEIN REQUIREMENT OF THE DOG.

By HOWARD B. LEWIS.

DEMONSTRATIONS BEARING ON COLORIMETRIC CREATININE AND NITROGEN DETERMINATIONS.

By OTTO FOLIN, E. A. DOISY, RICHARD BELL, and W. DENIS.

A NEW TEST FOR THE DETECTION OF OCCULT BLOOD IN STOOLS.‡

By W. G. LYLE.

THE PRODUCTION OF LACTIC ACID BY INTRAVENOUS INJECTION OF ALKALI.

By J. J. R. MACLEOD.

OBSERVATIONS ON THE FATE OF INOSITE IN THE DOG.

By ISIDOR GREENWALD and MORRIS WEISS.

ALKALINITY AND CARBONIC ACID IN SEA WATER.

By L. J. HENDERSON and E. J. COHN.

SOME EXPERIMENTS ON PROTEOLYTIC ACTIONS.‡

By E. M. FRANKEL.

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†Published in full in *J. Biol. Chem.*, 1917, xxix, 289.
‡Read by invitation.

THE KINETICS OF INVERTASE ACTION.*

By J. M. NELSON and W. C. VOSBURGH.

DEMONSTRATION OF A PERFUSION PUMP.**

By F. B. KINGSBURY.

VIRTUAL SUGAR.

By HUGH McGUIGAN.

THE DETERMINATION OF HEAVY METALS IN PHYSIOLOGICAL TISSUES AND FLUIDS.

By SAMUEL BOOKMAN.

A METABOLISM STUDY OF MANITOU (ALKALINE) MINERAL WATER.

By HELEN I. MATTILL and H. A. MATTILL.

THE REARING OF CHICKS UNDER LABORATORY CONDITIONS WITH ISOLATED FOOD SUBSTANCES.

By M. E. JAFFA and H. A. MATTILL.

THE INORGANIC COMPOSITION OF THORACIC DUCT LYMPH.†

By A. B. MACALLUM.

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*Read by invitation.

**See Kingsbury, F. B., *J. Biol. Chem.*, 1916–17, xxviii, 167.

†Read by title.

THE EXCRETION OF CREATINE AND CREATININE PARENTERALLY INTRODUCED.

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(Received for publication, December 6, 1916.)

The experiments of Folin (1) in 1906 led him to doubt that any metabolic relation exists between creatine and creatinine and for a time suppressed the principle laid down by Liebig (2) and generally accepted that urinary creatinine results from the metabolism of muscle creatine.

All experiments on the metabolism of creatine and creatinine in which the substances are fed are open to the criticism that absorption of the unchanged substances may never occur because of their destruction by microorganisms in the alimentary tract. Mellanby (3) in 1908 applied this criticism to Folin's experiment mentioned above, and in 1912 Twort and Mellanby (4) isolated from the feces an organism capable of decomposing creatine.

The results of experiments in which creatine or creatinine have been introduced parenterally are somewhat conflicting. Lefmann (5) obtained no evidence that creatine is converted to creatinine. Pekelharing and Van Hoogenhuyze (6) and Myers and Fine (7) working with rabbits noted an increased elimination of creatinine after the injection of creatine, while a part of the creatine reappeared unchanged after the administration of comparatively large amounts.

That creatine ingestion may be followed by increased creatinine excretion is further confirmed by several investigators, especially by Rose and Dimmitt (8) who fed large amounts, up to 20 gm. per day, of creatine to men. They obtained an unmistakable increase in creatinine output and recovered, unchanged in the urine, a part of the ingested creatine, as high as 75 per cent when the largest amounts were fed. After feeding creatinine, up to 16 gm. per day, 77 to 78 per cent was recovered in the urine unchanged while no creatine was excreted. These results lead Myers and Fine, and Rose and Dimmitt to conclude that an intimate physiological relation exists between creatine and creatinine.

The results of our experiments support this view. In order to avoid danger of bacterial decomposition of the materials in the intestine we injected sterilized solutions of creatine and creatinine subcutaneously. The 24 hour urines were analyzed daily by

the following methods: creatinine by Folin's method (9); creatine by the Folin-Benedict method (10); ammonia by Folin's method (11); urea by the method of Van Slyke and Cullen (12); total nitrogen by the Kjeldahl method.

The creatine used was prepared partly from dog muscle and partly from python muscle, the creatinine from urine by Benedict's method (13). The purity of the specimens was established by their nitrogen content. The creatine and creatinine were dissolved in 0.9 per cent sodium chloride solution and sterilized by boiling. The creatine solution after sterilizing gave a negative test for creatinine.

Experiments with Man.

A normal man, 28 years of age, weighing 80 kilos, and 1.8 meters in height served as subject. The diet was creatine-creatinine-free and consisted of bread, butter, sugar, starch pudding, chocolate, vegetables, and fruit. The character of the diet was constant but the quantity varied somewhat from day to day. The first injection was made on the 5th day after beginning the diet when the elimination of creatinine had become constant. The injections were made in the thigh or calf, the creatinine at one dose, and the creatine divided and given in thirteen portions at intervals of 30 minutes until all had been taken. Analyses of the first 4 days' urines are not included. The results follow.

Effect of Subcutaneous Injections of Creatine and Creatinine on Their Elimination in the Urine.

Day.	Volume.	Reaction to litmus.	Sp. gr.	Total N.	Urea N.	Ammonia N.	Creatinine N.	Creatine N.	Injected nitrogen recovered.	
									As creatine.	As creatinine.
	cc.			*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*per cent*	*per cent*
5	560	Acid.	1.025	5.81	4.50	0.43	0.52	0.000		
6	500	"	1.029	6.11	4.57	0.47	0.64	0.024	4	20
7	600	"	1.020	5.61	4.20	0.41	0.52	0.000		
8	576	Neutral.	1.023	6.53	5.16	0.46	0.56	0.0077	4	19
9	640	Acid.	1.020	5.88	4.59	0.41	0.52	0.000		
10	592	"	1.024	6.09	4.46	0.50	0.76	0.000	0	66

On the 6th day injected creatine containing 0.584 gm. of N.
" " 8th " " " " 0.194 " " "
" " 10th " " creatinine " 0.361 " " "

The fact that 19 to 20 per cent of the injected creatine nitrogen was recovered in the urine as creatinine is evidence that an intravital transformation of creatine to creatinine can occur. Since only 4 per cent of the injected creatine was excreted unchanged in the urine, 76 to 77 per cent of the injected nitrogen remains to be accounted for. Slight increases in total nitrogen, urea, and ammonia eliminated suggest a decomposition of the creatine or its transformation product creatinine. Our data on this point are not conclusive since there was a slight variation in the amount of protein ingested from day to day. Gottlieb and Stangassinger (14) have demonstrated the presence of creatine- and creatinine-destroying enzymes in autolyzing tissues, and Benedict and Osterberg (15), among others, have expressed the view that creatine may be destroyed in relatively large amounts in the normal mammal. That a part of the injected creatine was stored in the muscles is possible since Myers and Fine, and Folin and Denis (16) have noted an increase in muscle creatine after injection or absorption from the intestine of the same.

A large part of the injected creatinine (66 per cent) was recovered unchanged in the urine, without an increased creatine elimination. Rose and Dimmitt have argued that the creatine-creatinine transformation is not reversible in the body since creatine does not appear in the urine after ingestion of creatinine. This conclusion seems unwarranted to us since a small amount of creatine so formed might be completely destroyed or stored in the body. Myers and Fine have observed that the creatine content of rabbit muscle is increased by injection of creatinine into the intact animal.

Experiments with Rabbits.

Creatine and creatinine solutions were injected subcutaneously into rabbits as in the preceding experiment except that the creatine was given in a single dose instead of being divided into portions given at intervals. The results were substantially the same as with man. With the comparatively larger doses of creatine more was excreted unchanged and less converted into creatinine. The tables follow.

Rabbit I, Weight 1,980 Gm. Diet 300 Gm. of Carrots and 5 Gm. of Oats Daily.

Day.	Volume.	Reaction to litmus.	Sp. gr.	Total N.	Urea N.	Ammonia N.	Creatinine N.	Creatine N.	Injected nitrogen recovered.	
									As creatine.	As creatinine.
	cc.			*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*per cent*	*per cent*
1	180	Alkaline.	1.018	0.840	0.699	0.001	0.0240	0.000		
2	102	Acid.	1.028	0.720	0.561	0.003	0.0242	0.000		
3	96	Neutral.	1.026	0.701	0.548	0.001	0.0239	0.000		
4	150	Alkaline.	1.021	0.871	0.681	0.003	0.0473	0.000	0	77
5	120	"	1.020	0.699	0.502	0.000	0.0238	0.000		
6	101	"	1.029	0.740	0.560	0.007	0.0275	0.0156	51	11
7	150	"	1.025	0.816	0.642	0.004	0.0280	0.0129	43	13
8	190	"	1.012	0.806	0.630	0.004	0.0243	0.000		

On the 4th day injected creatinine containing 0.0297 gm. of N.
" " 6th " " creatine " 0.0302 " " "
" " 7th " " " " 0.0300 " " "

Rabbit II, Weight 1,810 Gm. Diet 300 Gm. of Carrots and 5 Gm. of Oats Daily.

Day.	Volume.	Reaction to litmus.	Sp. gr.	Total N.	Urea N.	Ammonia N.	Creatinine N.	Creatine N.	Injected nitrogen recovered.	
									As creatine.	As creatinine.
	cc.			*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*per cent*	*per cent*
1	160	Alkaline.	1.012	0.621	0.468	0.002	0.0244	0.000		
2	86	"	1.031	0.704	0.562	0.006	0.0234	0.000		
3	120	"	1.022	0.699	0.525	0.001	0.0230	0.000		
4	90	"	1.039	0.816	0.650	0.003	0.0460	0.000	0	79
5	130	Neutral.	1.016	0.760	0.545	0.004	0.0239	0.000		
6	96	Alkaline.	1.040	0.806	0.643	0.001	0.0268	0.0167	59	11
7	83	"	1.036	0.672	0.516	0.003	0.0270	0.0189	65	12
8	102	"	1.024	0.810	0.639	0.000	0.0236	0.000		

On the 4th day injected creatinine containing 0.0284 gm. of N.
" " 6th " " creatine " 0.0283 " " "
" " 7th " " " " 0.0288 " " "

SUMMARY.

Subcutaneous injections of creatine into rabbits and man were followed by increased urinary creatinine excretion which is further evidence of an intravital transformation of creatine to creatinine.

In man the larger part (76 to 77 per cent) of the creatine injected was destroyed or stored in the body.

The injection of creatinine was not followed by the excretion of creatine in the urine. This is not proof, however, that the creatine-creatinine mechanism is non-reversible.

Since writing the above a paper by Burns and Orr (17) has appeared in which they report no increased creatinine excretion after the ingestion of 0.5 gm. of creatine per day. They suggest that the increased creatinine elimination observed by Myers and Fine after the ingestion of creatine may have been due to a diuresis on the experimental days. In our experiments with man this criticism will not apply since the urine volume is no greater on the creatine than on the normal days.

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CHOLESTEROL AND CHOLESTEROL ESTERS IN HUMAN BLOOD.*

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Cholesterol occurs in practically all animal tissues and is therefore probably an important substance in the animal economy. It was one of the earliest biochemical substances discovered and because of its wide occurrence and also its relative stability it has been the subject of much investigation. Nevertheless there is little definite knowledge regarding its origin, fate, or function in living beings.

Cholesterol originates in the animal body mainly from the food, either as such in animal food, or in plant material as isomeric forms which the animal is able to change into cholesterol.[1] There is no satisfactory agreement among investigators as to whether it can be synthesized in the body. Dezani[2] has claimed that it is readily synthesized, while Gardner and Lander[3] consider its synthesis doubtful. Lifschütz[4] believes that it is formed from oleic acid. It seems likely, at any rate, that although synthesis is probable it is not an important factor in the supply to the animal. Cholesterol is absorbed from the intestine, increasing the blood cholesterol,[5–8] and during absorption is partly esterified with the fatty acids, the extent of combination being quite definite.[9]

As regards its fate in the body, there is some evidence that it may be burned[10] and also that it may be a source of the bile acids.[11] Ordinarily, however, the greater part, if not all, of the excess of cholesterol is excreted in the feces or by the skin after partly undergoing slight oxidation or reduction. It is excreted in the intestine practically entirely free, while in the skin secretions it appears almost entirely as esters of the fatty acids. In certain animals, notably the rabbit, there appears to be a limit to the power of excretion, and if large amounts are fed, it accumulates first in the blood, then in the organs such as the suprarenals, liver, and

* A report of this work was presented before the American Chemical Society in New York, September, 1916.

spleen, and also in the walls of the arteries, mainly as cholesterol esters, forming the anisotropic fat deposits.[5,6] Similar pathological deposits occur in human beings and there is an increasing tendency among clinicians to refer these and certain possibly resulting phenomena such as arteriosclerosis to excessive cholesterol in the diet. If cholesterol is excluded from the diet none is excreted, the organism apparently conserving its supply.[12]

The functions of cholesterol in the living organism are little understood. The one most widely discussed is that connected with the protective mechanisms of the body and particularly with its power to act as an antihemolytic against such substances as saponin,[13] tetanolysin,[14] etc. The free cholesterol alone is effective in this reaction, the esters having no antihemolytic power,[15] the reason being that the cholesterol probably forms an ester-like compound with the poison through the free hydroxyl group. Lecithin has been found to act antagonistically to cholesterol in this function by activating the lysin.[16] Cholesterol has been found by Robertson[17] to accelerate the growth of tumors in mice and also to stimulate the growth of rats,[18] in both of which lecithin has been found to have the opposite effect although to a much less degree. It has been found by Wacker[29] that cholesterol accumulates in the body fat with increasing age, and Robertson[30] has advanced the view, which is shared by other recent workers,[31] that this accumulation of cholesterol may account for the increased incidence of tumors with advancing age. A third function of cholesterol and one to which little attention has been paid heretofore is the part which it plays in the metabolism of the fatty acids. The fatty acid esters of cholesterol are almost as generally distributed in the animal body as cholesterol itself. Moreover, it has been shown that during the absorption of cholesterol from the intestine considerable quantities of esters are formed.[1,9] It has been claimed by several investigators that the cholesterol of the blood is increased during the absorption of fat free from cholesterol,[19,20] although this finding has not been confirmed by recent work.[28] Further evidence of the participation of cholesterol in fat metabolism is furnished by recent investigations[21,22] on the partition of the lipoids in normal and pathological blood, in which it was shown that cholesterol bears a strikingly constant relation to the other blood lipoids, a relation which was maintained even in a severe diabetic lipemia.[22]

It is desirable in the study of most of these functions and particularly of the part which cholesterol takes in fat metabolism to know the extent to which it is combined with the fatty acids, whether there is normally a balance between the free and bound cholesterol, and whether this balance is maintained in abnormal conditions. A few results have been reported to show that there is in most animals a definite balance between free and bound cholesterol in normal blood[1,23,24] and that this balance may be disturbed in certain abnormal conditions.[23] In order to confirm and extend these findings for human blood the following study was undertaken. The blood samples examined were from a number of normal men and women and from hospital patients of both sexes, the lipoid values of

whose blood have already been reported.[21, 22] In addition there were a number of others obtained mainly from the wards of the Massachusetts General Hospital through the kindness of Dr. W. Denis.

The methods for cholesterol and cholesterol esters have been reported recently.[25, 26] They consist in determinations of these substances in extracts of the blood before and after precipitation of the free cholesterol with digitonin. For total cholesterol 10 cc. of the extract were ordinarily used and 20 cc. for the cholesterol esters. The results are given in the tables.

TABLE I.

Cholesterol and Cholesterol Esters in Human Blood. Mg. per 100 Cc.

Normal.				Pathological.			
Men.	Total.	As ester.		Men.	Total.	As ester.	
	mg.	*mg.*	*per cent*		*mg.*	*mg.*	*per cent*
	193	69	35.7	Diabetes.	332	155	46.6
	201	65	32.3		316	156	49.3
	180	59	32.8		197	71	36.0
	180	62	34.4		299	135	45.1
	178	60	33.7		344	150	43.6
				Pernicious anemia.	156	62	39.7
Average.....	.....	.....	33.8	Hemophilia.	156	52	33.3
Women.				Women.			
	195	60	30.8				
	177	60	33.9	Diabetes.	382	203	53.1
	197	61	30.9	"	352	199	56.5
	184	60	32.6	Pernicious anemia.	166	63	38.0
	208	87	41.8	" "	156	62	39.7
	205	61	29.7	Carcinoma.	212	75	35.3
				"	180	42	23.3
Average.....	.....	.....	33.3	Uremia.	212	72	34.0
				Hyperthyroidism.	238	81	32.0
				Typhoid.	185	82	44.3
				Goiter.	247	83	33.6
				Asthma.	172	63	36.6
				Pregnancy.	291	160	54.9
				"	181	85	47.0
				"	250	150	60.0

TABLE II.

Cholesterol and Cholesterol Esters in Human Blood Plasma. Mg. per 100 Cc.

Normal.				Pathological.			
Men.	Total.	As ester.		Men.	Total.	As ester.	
	mg.	*mg.*	*per cent*		*mg.*	*mg.*	*per cent*
	202	120	59.4	Diabetes.	250	102	40.8
	207	116	56.1		282	170	60.3
	208	127	61.0		396	208	52.5
	200	117	58.5		454	272	59.9
	245	120	49.0		470	253	53.8
	180	117	65.0		476	223	46.8
	291	164	56.3		508	218	42.9
	190	90	47.4		538	250	46.4
	192	109	56.7		577	240	41.6
	210	117	55.7		915	505	55.1
	198	112	56.5		1,060	594	56.0
	196	117	59.6	Pernicious anemia.	150	100	66.6
	208	128	61.5		149	66	44.3
	203	123	60.6	Nephritis.	161	58	36.0
	220	150	68.2		170	86	50.0
	195	117	60.0		165	74	44.8
	221	101	45.7		195	115	58.9
				Carcinoma.	178	64	36.0
Average.....			57.5	Hemophilia.	158	103	65.2
				Addison's disease.	252	130	51.5
Women.				Women.			
	227	126	55.5	Diabetes.	182	116	63.7
	210	133	63.3		272	166	61.0
	247	137	55.4		275	180	65.5
	217	152	70.0		356	211	59.2
	195	121	62.0		455	250	54.9
	140	92	65.7		559	358	64.0
	163	83	50.9		593	356	60.0
	175	102	58.2	Pernicious anemia.	149	66	44.2
	152	86	56.5		170	106	62.0
					170	110	64.7
Average.....			59.7		173	110	63.6
					150	88	58.6
					160	125	78.0
					140	90	64.3
				Carcinoma.	181	51	28.1
					187	78	41.5
					233	82	35.2
					240	86	36.0
				Hyperthyroidism.	199	62	31.1
				Anemia with leukemia.	210	106	50.6
				Uremia.	255	78	30.6

RESULTS AND DISCUSSION.

In normal whole blood the average percentage of combined cholesterol was found to be 33.8 for men, with variations from 32 to 36; in women 33.3 with variations from 30 to 42 per cent.

In normal plasma the average percentage of cholesterol combined as ester in men was found to be 57.5 with variations from 46 to 68; for women, average 59.7 with variations from 51 to 70 per cent. These values agree very well with those reported in the literature.[23, 24] The variations from the average, generally not more than 15 per cent, are the same as those found for other blood lipoids and indicate that these two substances are subject to the same sort of regulation.

In pathological conditions the relation between free and bound cholesterol remains normal in most instances. Even in those cases (diabetes) where the total cholesterol is four or five times the normal value the relation holds constant. Low values for cholesterol esters in the plasma were found in most cases of nephritis and in all the cases of carcinoma examined. The values for diabetic plasma, although within normal limits, are in general definitely below the average, in contrast to the corresponding values in whole blood which are distinctly high. In whole blood the only abnormal values for cholesterol esters, aside from diabetes, were found in pregnancy where they were definitely high, as has also been reported by Herrmann and Neumann.[27]

The constancy of the relationship between free and bound cholesterol in normal individuals indicates that with cholesterol esters as with the other lipoids there is an efficient regulation, and the fact that there is so little variation from the normal relationship in abnormal conditions emphasizes what has been found for most other blood constituents—that the metabolic habits of the individual are not easily upset even by severe disease.

The percentage values for free and bound cholesterol in normal whole blood and plasma bear out the assumption generally made that there is little if any bound cholesterol in the corpuscles. However, in certain of the abnormal conditions noted above, as, for example, diabetes, the relatively low values in plasma together with the high values in whole blood indicate that in some cases the corpuscles may contain combined cholesterol. Further investigation is needed to confirm these indications.

The balance between cholesterol and its esters and therefore between cholesterol esters and the other blood lipoids, gives further ground for the assumption previously made that cholesterol takes an active part in fat metabolism. The possibility cannot of course at present be excluded that this part is secondary to the function of the fatty acids in cholesterol metabolism and that the esters constitute a convenient form in which the excess of cholesterol may be carried in the blood or stored in the tissues (cholesterol esters appear to be the main constituents of the deposits of "anisotropic fats"), but the fact that the blood cholesterol may increase to several times normal (diabetes) or be considerably below normal (anemia) without disturbing the balance speaks against this latter assumption. Determinations of the balance between cholesterol and its esters during fat absorption should give valuable information on this question.

SUMMARY.

In normal human blood there was found a constant relation between free cholesterol and cholesterol esters. In whole blood the average percentage of cholesterol in combination as esters was found to be about 33.5 per cent, in plasma 58 per cent of the total cholesterol. The variation from the average in individuals was about 15 per cent of the average. There was no notable difference in these values between men and women.

In pathological conditions the relation between free and bound cholesterol was found normal in all cases except carcinoma and most cases of nephritis, in both of which the percentage combined as ester was low.

In pregnancy the values for cholesterol esters were high.

The percentage values for free and bound cholesterol in whole blood and plasma in almost all the samples examined bear out the claim that there are few if any cholesterol esters in the corpuscles.

The constant relation between free and bound cholesterol gives further support to the assumption that cholesterol takes an active part in fat metabolism.

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A NOTE ON BENEDICT'S METHOD FOR THE ESTIMATION OF TOTAL SULFUR IN URINE.

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(Received for publication, December 6, 1916.)

The determination of the amount of total sulfur, and consequently the "neutral" sulfur content of urine, demands an accurate method, if any reliance is to be placed upon the values for the latter factor. Folin (1), Benedict (2), Ritson (3), Gill and Grindley (4), and others (5) have laid down procedures for the oxidation and estimation of the "neutral sulfur compounds" in urine. Of all of these only Benedict's method seems to have survived the storm of criticism. In principle it depends upon the oxidation of the urine with copper nitrate.

Besides the proof offered by its originator, and the fact that it is included by several authors in their text-books there is additional evidence (6) that Benedict's method is satisfactory. Schmidt (7), in whose hands it gave concordant results, found that it compared favorably with Folin's method. He had some slight difficulty, however, and says: "Any slight loss due to spattering, which may sometimes take place in oxidizing with copper nitrate, can be prevented by covering the evaporating dish with a watch-glass." Such a procedure may prevent any loss by spattering, but does not prevent spattering.

Denis (8) attempted to use Benedict's method but this attempt she says, ". . . . resulted in utter failure, as, owing to the violent decrepitation and spattering produced at the beginning of ignition by the sodium or potassium chlorate employed, mechanical loss invariably resulted in every one of the forty attempts made. No amount of care in heating seemed to lessen the spattering." She offered no figures to substantiate the statements, as Benedict (9) himself notes, and, instead of trying to overcome the spattering, proceeded to modify the original method.

In the course of work involving about 100 estimations of total sulfur by the use of Benedict's method, it was found that a 3-heat electric hot plate could be employed to avoid spattering or crepitation. If the procedure later outlined is followed these difficulties disappear. Consistent results can be obtained, as the few following typical duplicate analyses of 10 cc. samples of urine indicate:

$BaSO_4$ *mg.*	Difference. *mg.*
71.7 71.6	0.1
32.2 31.7	0.5
18.6 18.3	0.3
42.6 42.7	0.1
50.3 49.8	0.5
48.6 48.5	0.1
56.4 56.0	0.4

We have used the method as follows: 10 cc. of urine are delivered into a new 150 cc. casserole. Any trace of urine on the side of the dish is washed down with 10 cc. of the Benedict sulfur reagent. The casserole is then placed on the hot plate plugged in "low," without further attention; or if it is desired to hasten the process but sacrifice some time in watching, one can plug in "medium heat." In about 45 minutes to an hour the contents of the casserole will be evaporated to dryness and the heat may or may not be high enough to cause the nitrate to "go off." In any event the casserole is removed from the hot plate, put directly over the Bunsen, and heated to the fullest extent of the burner for 10 or 12 minutes to drive off all NO_2 and decompose all chlorate. The dish is cooled, the contents are then dissolved in 15 cc. of 1 : 4

HCl, and diluted with water to about 100 cc. The sulfates present are precipitated with 10 cc. of 10 per cent barium chloride from a dropping tube.

After a good many determinations it may be necessary to filter after dissolving in HCl, as the porcelain may begin to chip off from the heat.

The use of the hot plate does away with all watching and care during evaporation. The heat is sufficient for the oxidation. The number of analyses that can be carried out simultaneously is limited only by the size of the hot plate.

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THE CHEMICAL COMPOSITION OF THE PLACENTA.

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(Received for publication, December 5, 1916.)

It is well known that no continuity exists between the maternal and fetal circulations and that interchanges of nutritive material and waste products take place by diffusion through the capillary walls and other membranes at the place or places of contact between the uterus and the fetus.

In the discoidal placenta of the human race the maternal and fetal parts are so closely related that it is impossible to separate them quantitatively, and this placenta is consequently less suitable for some experimental purposes. In the multiple placenta of the cow, the fetal parts are readily and completely separated from the maternal parts and for this reason this placenta was chosen for the present work which was carried out for the purpose of determining the constituents of these two portions of the placenta.

It may be in order to give here a short description of the placental mechanism in the uterus of the cow.[1] With the development of the fetus the uterus undergoes important anatomical and physiological modifications. When gestation has commenced the surface of the maternal caruncles, previously smooth, becomes convex, and is covered with reticulate processes which border the crypts and give it a finely cribbled appearance. The largest are found in the body of the uterus and they become smaller as they approach the extremity of the cornua. In shape they resemble mushrooms, and their color is dark yellow. They constitute the maternal portion of the placenta. The villi of the chorion are developed and agglomerated in larger numbers at certain points of its surface to constitute a multiple polycotyledonary or tufted placenta which is composed in this way of sixty to eighty placental or fetal cotyledons. They are bright red, oval, concave patches 0.4 to 0.6 inches thick and correspond to the maternal cotyledons of the uterus into which they are received.

[1] Craig, J. F., Fleming's Veterinary Obstetrics, London, 1912.

Two stages of pregnancy were selected for the collection of samples from cows, the first with fetuses 3 to 4 months old and the second with almost mature fetuses 8 to 9 months old. In both instances twelve pregnant uteri were employed. These were taken at random on different days, from several hundred pregnant uteri, and the final samples, therefore, are good average representatives of the two stages of pregnancy. Twelve cotyledons from each uterus were removed, carefully trimmed, and the fetal and uterine portions separated by hand. This is an easy matter in this case as the two parts pull apart readily. The fetal and maternal placentulas were again trimmed and weighed, finely minced, and dried *in vacuo* at 35–50°C. The desiccated material was coarsely ground and extracted with petroleum ether in a Soxhlet apparatus.

The maternal cotyledons are grayish yellow in color, while the young fetal cotyledons are pale pink and the older ones dark red. The former are considerably larger than the latter but the sizes of both are in direct proportion to the age and weight of the fetus.

The petroleum ether-soluble substances were obtained by extraction of the desiccated materials with this solvent. After extraction, the liquids were filtered and evaporated on a water bath with a current of air and finally dried *in vacuo* at 50°C. to constant weight. During extraction it was noticed that the uterine placenta gave a bright yellow color to the petroleum ether and the fetal placenta a deep orange color, indicating that both placentas contain distinct coloring matters soluble in the usual fat solvents. After evaporation the products maintained their colors. They were all semisolid and had a strong lecithin odor. Total phosphoric acid was determined on these samples and the amounts of phosphatides estimated as lecithin by multiplying the P_2O_5 figures by 11.24.

Table I gives the average weight of the maternal cotyledons and fetal placentulas, the percentage of moisture and petroleum ether-soluble material in the fresh tissue, and the yield of desiccated fat-free material, also the percentage of phosphatides calculated as lecithin in the fresh tissue. On the desiccated fat-free material was determined moisture, total nitrogen, and ash. The mineral constituents of the four samples were also determined.

TABLE I.

	Fresh tissue.						Desiccated fat-free material.										
	Average weight.	Moisture.	Petroleum ether-soluble substances.	P_4O_5 in petroleum ether-soluble substances.	Phosphatides calculated as lecithin in fresh tissue, $P_4O_5 \times 11.24$.	Desiccated fat-free material.	Moisture.	Ash.	Total nitrogen.	Protein ($N \times 6.25$).	Phosphoric acid (P_4O_5).	Sulfuric acid (SO_3).	Chlorine (Cl).	Potassium (K).	Sodium (Na).	Iron (Fe).	Calcium (Ca).	Magnesium (Mg).
	gm.	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Maternal placentulas (uterine cotyledons) 3 to 4 months old	13.6			2.10	0.34	15.35	5.55	6.50	13.17	82.30	3.95	1.85	0.17	1.51	0.85	0.014	0.179	0.115
Dry basis								6.88	13.95	87.15	4.18	1.96	0.18	1.60	0.90	0.015	0.189	0.122
Original basis		83.20	1.45*					1.06	2.14	13.38	0.642	0.310	0.028	0.246	0.138	0.002	0.029	0.019
Maternal placentulas (uterine cotyledons) 7 to 9 months old	36.6			2.22	0.37	16.52	4.45	6.38	13.41	83.81	3.77	1.79	0.15	1.71	0.71	0.02	0.179	0.127
Dry basis								6.68	14.04	87.75	3.96	1.87	0.157	1.79	0.74	0.021	0.187	0.133
Original basis		82.00	1.48*					1.10	2.32	14.50	0.654	0.309	0.026	0.296	0.122	0.003	0.031	0.022
Fetal placentulas (fetal cotyledons) 3 to 4 months old	10.4			2.50	0.19	10.91	7.05	10.25	12.03	75.19	3.92	1.56	2.06	1.87	1.73	0.028	0.457	0.109
Dry basis								11.03	12.94	80.89	4.22	1.68	2.22	2.02	1.86	0.030	0.492	0.117
Original basis		88.40	0.69†					1.20	1.41	8.83	0.461	0.183	0.242	0.220	0.203	0.003	0.054	0.013
Fetal placentulas (fetal cotyledons) 7 to 9 months old	27.6			1.51	0.14	14.96	5.05	6.95	13.18	82.37	3.65	1.62	0.49	1.44	0.96	0.063	0.159	0.120
Dry basis								7.32	13.89	86.70	3.84	1.71	0.52	1.52	1.01	0.066	0.167	0.126
Original basis		84.20	0.84†					1.09	2.08	12.97	0.574	0.256	0.078	0.228	0.151	0.010	0.025	0.019

* Yellow; semisolid; lecithin odor.

† Dark orange; semisolid; lecithin odor.

Total nitrogen determinations were made according to the official Gunning-Kjeldahl method. The phosphoric acid was determined both in the petroleum ether-soluble substance and in the desiccated fat-free material by heating 1 gm. of the substance with 2 cc. of sulfuric acid and several small portions of nitric acid, until oxidation was complete. The clear liquid was diluted with water and the phosphorus pentoxide determined by the official volumetric method.

The sulfur, iron, calcium, and magnesium determinations were made in the usual way after previous fusion of the desiccated fat-free substance with fusion mixture. The sodium, potassium, and chlorine were determined on the ash which was incinerated at a temperature below dull red heat.

The tabulated results show that the maternal cotyledons contain less moisture and more protein and fats, phosphatides, etc., than their corresponding placentulas. The older uterine cotyledons contain more potassium and less sodium than the younger, but otherwise the variations in the mineral constituents are comparatively slight at the two selected stages of pregnancy.

The young fetal placenta contains much more chlorine and considerably more sodium and calcium and less of the other inorganic constituents than the older fetal placenta. It is evident, therefore, that chlorine, sodium, and calcium are of vital importance in early fetal life. Later, during the rapid growth periods, the amounts of these three elements diminish while the quantities of phosphorus, sulfur, potassium, iron, and magnesia increase.

The 3 to 4 months old fetal placentulas, which are of a pale pink color, contain only about one-third the quantity of iron that is present in the older and dark red fetal placentulas. This indicates that the amount of hemoglobin in the fetal blood is small during the earlier stages of pregnancy, but increases in direct proportion to the growth of the fetus.

All four samples gave negative tests for epinephrin and iodine when lean meat was employed for control analyses.

5 gm. portions of the dry powders were macerated at room temperature with 50 cc. of water, and iodine solution was added until a permanent yellow color remained. After standing for some time the uterine liquids developed a pronounced port wine coloration similar to that produced by addition of iodine to glycogen solutions. The color appeared first in the finer particles of the sediment and was gradually yielded to the liquid. This did not occur in the young fetal extract, which remained light yellow. The old fetal maceration was of a dark red color which prevented

observation. This test, therefore, seems to indicate that glycogen is stored in the uterine portion of the placenta during pregnancy but not in the fetal part. No attempts were made to separate or identify the glycogen further.

In an attempt to separate the liquid from the tissue, portions of the finely minced fresh substances, both fetal and uterine, were centrifuged in a large powerful machine for 1½ hours at a speed of 3,200 revolutions per minute. The cells, however, hold the liquid very firmly as it was possible to obtain only a few cc. from 200 gm. portions of the various samples. The liquids were all faintly acid to litmus, opalescent, and light orange-colored from the uterine placentas. The older fetal placenta yielded dark red, and the younger yellowish pink fluids.

SUMMARY.

The constituents of the uterine placenta seem to remain comparatively uniform throughout gestation, while the fetal placenta varies considerably in composition at different stages of pregnancy. Analytical data of the two portions of the placenta are given and the results discussed.

THE ESTIMATION OF HYDROCYANIC ACID AND THE PROBABLE FORM IN WHICH IT OCCURS IN SORGHUM VULGARE.

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INTRODUCTION.

The biochemistry of cyanogen compounds has received a considerable amount of attention, not only because of their practical importance in such substances as bitter almonds, linseed cake, fodder grasses, etc., but also because of the theoretical interest which attaches to their probable function in plants. From either viewpoint, facts of first importance to be ascertained are the nature of the cyanogenetic compounds, means of estimating them quantitatively, their situation in the plant, and their reaction to mechanical and chemical treatment of the plant. Although the first two points have received more attention than the others, they are still far from being answered satisfactorily. It is the object of the present paper to describe some experiments on *Sorghum vulgare* bearing on these questions.

Concerning the form in which prussic acid occurs in plants, it is generally believed that it exists free in but few cases, being usually combined as glucosides. About eight such glucosides have been isolated and identified. The nature of the cyanide body, however, is still unknown in the great majority of the plants which are known to contain the cyanide group. Only a few cases are on record where the evidence pointed definitely to the existence of uncombined hydrocyanic acid. Treub[1] found it "free or in a very loosely combined state" in *Pangium edule*, a tropical

[1] Treub, M., Sur la localisation, le transport, et le rôle de l'acide cyanhydrique dans le *Pangium edule*, *Ann. Jardin Bot. Buitenzorg*, 1896, xiii, 1.

tree containing as high as 0.3 per cent of the dry weight of the leaves as hydrocyanic acid. Greshoff,[2] with the same plant, came to similar conclusions. Later Peche,[3] in working out his microchemical test for cyanide, found it free or "in a very labile form" in both *Pangium edule* and in *Prunus laurocerasus*. Moore[4] believed that he had free hydrocyanic acid in cassava. The writer, however, questions this interpretation of his results, as will be discussed later.

Concerning the quantitative estimation of cyanogenetic compounds, it has usually been accomplished by the estimation of the cyanide itself, since it is impossible to extract and crystallize out the glucosides quantitatively. And since in most instances the hydrocyanic acid itself, and not the glucoside, is the critical element under observation, the investigator is content to determine the total cyanide present and disregard any other bodies which may be combined with it. A glucoside linkage is usually assumed, and the plant tissues treated with a view to hydrolyzing this glucoside and setting free the hydrocyanic acid. This is done (1) by autolysis (maceration with water), (2) by added enzyme (emulsin), or (3) by boiling with acids. Autolysis has been used frequently; in fact, the first discovery of hydrocyanic acid in the plant world by Böhm in 1803[5] was in water which had been in contact with crushed bitter almonds. The writer used it in his first studies on the dhurrin in sorghum.[6] Autolysis depends upon the coincident existence of glucoside and glucosidase in the same tissues, but not in contact with each other until the tissues are crushed. Since in a given tissue the enzyme must be perfectly specific for its accompanying glucoside, this method of hydrolysis, where applicable, is very satisfactory. Emulsin seems to act on all cyanogenetic glucosides; and, although its action is less rapid

[2] Greshoff, M., Distribution of prussic acid in the vegetable kingdom, *Report Brit. Assn., York*, 1906, 138.

[3] Peche, K., Mikrochemisher Nachweis der Cyanwasserstoffsäure in Prunus laurocerasus L., *Sitzungsb. k. Akad. Wien*, 1912, cxxi, 33.

[4] Moore, C. C., Cassava: its content of hydrocyanic acid and starch and other properties, *U. S. Dept. Agric., Bureau of Chemistry, Bull. 106*, 1907, 1.

[5] Böhm, *Neues allgemein. J. Chem.*, 1803 (original not consulted).

[6] Willaman, J. J., and West, R. M., Notes on the hydrocyanic-acid content of sorghum, *J. Agric. Research*, 1915, iv, 179.

than that of the autolyzing enzymes, it is nevertheless a highly effective agent in bringing about the hydrolysis of various glucosides. Acid hydrolysis in most cases gives the same final products as does enzyme hydrolysis, but probably does not go to completion or follows a different course. Caldwell and Courtauld,[7] using a 10 per cent solution of amygdalin and normal hydrochloric acid, found only 85 per cent hydrolysis in 200 hours at 60°C., and 92 per cent in 16 hours at 80°C. Walker and Krieble[8] obtained slow and incomplete hydrolysis with hydrochloric and sulfuric, and none with oxalic or trichloroacetic acid. Recently several investigators[9, 10, 11] have used 5 per cent sulfuric or 5 per cent tartaric acid. They judged the completeness of their hydrolysis only by the failure of further hydrocyanic acid to distill over. That this may be a false assumption, at least in the case of certain plants, will be brought out in the following experiments. The writer and West, in the 1915 work on sorghum at the Minnesota Experiment Station,[12] following the directions of Viehoever and Johns, ground the leaves in a food chopper and then distilled from 5 per cent tartaric acid. No further cyanide was obtained after an hour's distillation. That cyanide obtained in this way is not the product of acid hydrolysis, but of enzyme hydrolysis taking place previous to the addition of the acid, is in part the object of the present paper to show.

The success of any method for the determination of prussic acid depends not only on the complete hydrolysis of the glucoside, but also on the complete removal of the cyanide from the hydrolysate. A thorough discussion of this latter phase of the question was given by Alsberg and Black.[10] In their experiments with

[7] Caldwell, R. J., and Courtauld, S. L., The hydrolysis of amygdalin by acids, *J. Chem. Soc.*, 1907, xci, 666.

[8] Walker, J. W., and Krieble, V. K., The hydrolysis of amygdalin by acids, *J. Chem. Soc.*, 1909, xcv, 1369.

[9] Viehoever, A., and Johns, C. O., On the determination of small quantities of hydrocyanic acid, *J. Am. Chem. Soc.*, 1914, xxxvii, 601.

[10] Alsberg, C. L., and Black, O. F., The separation of autogenous and added hydrocyanic acid from certain plant tissues and its disappearance during maceration, *J. Biol. Chem.*, 1916, xxv, 133.

[11] Viehoever, A., Johns, C. O., and Alsberg, C. L., Cyanogenesis in plants. Studies on *Tridens flavus* (tall red top), *J. Biol. Chem.*, 1916, xxv, 141.

[12] Willaman and West, Effect of climatic factors on the hydrocyanic acid content of sorghum, *J. Agric. Research*, 1916, vi, 261.

several cyanogenetic plants they noticed a loss of hydrocyanic acid when the macerated tissue was allowed to stand some hours before distilling from acid. Also, added cyanide was lost in the same way. A non-cyanogenetic plant, *Sambucus canadensis*, did not have this power. They proved that the phenomenon was not due to enzymes, and suggested that there was a chemical reaction between the hydrocyanic acid and some other substances in the tissues, probably aldehydes. In another paper by Viehoever, Johns, and Alsberg,[11] retention of hydrocyanic acid on maceration was reported in *Tridens flavus;* maceration in presence of tartaric acid prevented this retention. Henry and Auld[13] devised a scheme which was designed to prevent retention of cyanide by the macerated tissue. They extracted the material with alcohol, evaporated the alcohol, then took up with water, and distilled with 2 per cent HCl. The cyanogenetic compounds were no doubt removed in this way, but whether they were all hydrolyzed by the acid is doubtful.

It is evident that there is considerable cause for questioning our knowledge of the actual amount of hydrocyanic acid present in various plants and the conditions in which it exists there. Most workers in this field have not taken into account (1) the possible incomplete liberation of hydrocyanic acid from its compounds, (2) the possible incomplete removal of the acid by distillation, and (3) the existence of various states of combination between the hydrocyanic acid and other constituents of the plants. The following experiments deal with these three points in the cyanogenesis of sorghum.

EXPERIMENTAL.

Material and Method.

The sorghum used in these experiments was of the Early Amber variety grown on the plots of the Minnesota Agricultural Experiment Station. Most of the samples were taken between the stages of full bloom and maturity, when the plants were 6 feet high or over. The leaves only were used.

The distilled hydrocyanic acid was collected in an excess of sodium hydroxide, and determined colorimetrically by the Prussian blue method of Viehoever and Johns.[9]

[13] Henry, T. A., and Auld, S. J. M., The occurrence of cyanogenetic glucosides in feedingstuffs, *J. Soc. Chem. Ind.*, 1908, xxvii, 428.

The procedure with some samples furnished illustrations for more than one factor under observation. Hence in the tables considerable duplication of laboratory numbers will be noticed. Instead of describing and discussing the procedure followed for each sample of leaves in chronological order and noting the various points observed, each factor (as acid hydrolysis, autolysis, etc.) will be taken up by itself, and in the tables reference will be made to the experiments from which the data were obtained.

Comparison of Acid and Enzyme Hydrolysis.

In order to determine the efficiency of various acids as hydrolyzing agents for cyanogenetic glucosides, the trials described in Table I were carried out, using amygdalin as the glucoside. Hydrolysis by emulsin was also performed for comparison.

TABLE I.

No.	Method of hydrolysis.	HCN		
		Present.	Recovered.	
		mg.	*mg.*	*per cent*
53	34.4 mg. amygdalin distilled 1¼ hrs. with 5 per cent tartaric	2.00	0.00	0.0
54	34.4 " " distilled 1¼ hrs. with 5 per cent H_2SO_4	2.00	0.00	0.0
55	200 " " in 40 cc. water, 100 mg. emulsin, 24 hrs. at 45°C.	10.56	10.40	98.5
56a	200 " " distilled 2¼ hrs., 200 cc. 5 per cent H_2SO_4	10.56	0.40	3.8
56b	200 " " distilled 2¼ hrs., 200 cc. 5 per cent HNO_3	10.56	0.00	0.0
56c	200 " " distilled 2¼ hrs., 200 cc. 5 per cent tartaric	10.56	0.00	0.0
57	200 " " boiled 24 hrs. with 200 cc. 5 per cent H_2SO_4*	10.56	0.70	6.7
58a	50 " " 10 mg. emulsin, 47°C. 4 hrs.	2.64	2.20	83.3
58b	50 " " 5 mg. emulsin, 47°C. 4 hrs.	2.64	1.40	53.0
73	200 " " 200 cc. 2 per cent HCl, 72 hrs. boiling	10.56	2.80	26.5

*The material was boiled under a reflux connected with bulbs of NaOH. After the cyanide had been determined, the ammonia was distilled off; 1.0 mg. of N as NH_3 was obtained, corresponding to 1.9 mg. of HCN.

The results show that no acid is at all efficient for the hydrolysis of amygdalin. As evidenced in No. 57, considerable of the hydrocyanic acid in the glucoside is set free as ammonia by strong acids, according to the well known mandelonitrite decomposition. Emulsin proved a very efficient hydrolyzing agent. How far we are safe in assuming that what is true for amygdalin is also true for dhurrin, we cannot say at present. However, the succeeding experiments will show that dhurrin is probably not hydrolyzed by acids to any greater extent than is amygdalin.

Effect of Autolysis.

In Table II are collected the data obtained in the various experiments dealing with the autolysis of the macerated sorghum leaves. The procedure in general was to grind the leaves in a food chopper, and then put them into 300 cc. of water for autolysis, or into 300 cc. of 5 per cent tartaric acid for distillation. It was not expected that the acid would hydrolyze any dhurrin; it merely furnished an acid medium from which to distill the hydrocyanic acid present in the tissues from any source whatsoever, and prevented enzyme action. In order to preclude entirely any chance for enzyme action on the dhurrin, in some cases the leaves were moistened with the tartaric acid before grinding, and then were ground directly into more of the acid. In the tables this method is called "grinding with tartaric," and is used as a check determination in most cases to judge the effect of autolysis.

In Nos. 60 and 62 there is a marked increase in hydrocyanic acid after hydrolysis; there is no increase in Nos. 70 and 65, where no tartaric acid was added before distilling. This is the reverse of what was suggested by Viehoever, Johns, and Alsberg.[14] They did not offer an explanation of why the addition of acid after the period of autolysis should affect the retention of cyanide. In Nos. 87 and 84, the check was ground with tartaric; the other sample had a chance to autolyze from the moment the tissues were ruptured till the macerated mass was heated to the killing temperature of the enzyme, probably 10 minutes. This was evidently sufficient time for considerable hydrolysis to take place,

[14] Viehoever, Johns, and Alsberg, Cyanogenesis in plants. Studies on *Tridens flavus* (tall red top), *J. Biol. Chem.*, 1916, xxv, 142.

but insufficient for much "retaining" action of the tissues on the hydrocyanic acid. In comparison to this, Nos. 87 and 85 show complete retention in 16 hours' autolysis. Two examples of 4 hour autolysis are given in Nos. 88a and 88b, and Nos. 91a and 91b. There is a marked increase in both cases.

It is apparent from these data that the hydrolysis of dhurrin in sorghum leaves by the action of the contained glucosidase takes place rapidly; it is probably completed in a few hours, perhaps in a few minutes. Auld[15] found that when linseed cake was auto-

TABLE II.

The Effect of Autolysis of Sorghum Leaves for Various Lengths of Time, on the Yield of Hydrocyanic Acid.

Nos.	Conditions of autolysis.	Method of determining HCN.	HCN per 100 gm. of dry matter.	
			Before autolysis.	After autolysis.
			mg.	*mg.*
60 and 62	24 hrs., 47°, 300 cc.	Distilled from 5 per cent tartaric.	7.12	12.25
70 " 65	24 " 47°, 300 "	Distilled from water.	12.40	12.40
87 " 84	10 min., previous to reaching the boiling point.	" " "	1.74	10.45
87 " 85	16 hrs., 47°, 300 cc.	" " "	1.74	0.00
88a " 88b	4 " 23°, 300 "	Check ground with 5 per cent tartaric; autolyzed sample distilled from water.	0.00	5.51
91a " 91b	4 " 23°, 300 "		0.00	7.16

lyzed in water at 37°C., most of the hydrocyanic acid was liberated in 15 minutes, and all of it in 6 hours.

In Moore's investigation of cassava[4] he ground the tissues to a pulp, placed it in a retort, and slowly distilled for 2 hours. Most of the cyanide passed over in the early stages. He says: "From this fact and the nature of the results obtained there is no reason to believe that the figures given represent other than the HCN existing in the free state." He got no increase in cyanide by the

[15] Auld, S. J. M., Formation of prussic acid from linseed cake and other feedingstuffs, *J. Southeast Agric. College Wye*, 1911, no. 20, 289.

use of sulfuric acid. Now, if autolysis in cassava proceeds at anything like the rate it does in sorghum, there was time in Moore's process for considerable liberation of cyanide before the mass of tissue reached a temperature fatal to the enzymes. There may be free hydrocyanic acid in cassava; but it is the writer's conviction that the above process gives considerably more than the non-glucosidic hydrocyanic acid.

In the same way, when Peche[3] concluded that he had free hydrocyanic acid when a section of the tissue gave a reduction of mercurous nitrate, it is possible that the act of sectioning brought glucoside and enzyme in contact for a time sufficient to set free enough hydrocyanic acid to give a test. By the time these facts on sorghum were ascertained, material was not available for making a more thorough study of this rate of autolysis. The writer believes, however, that for each plant species an optimum time for autolysis can be found during which a maximum yield of hydrocyanic acid can be obtained. This may not represent all the cyanide present; it probably is the difference between the total amount set free and the amount retained by the macerated tissue. What may be the nature of this retention is not known; it is suggested that, if aldehydes are responsible, some substance may be found which, added to the macerated tissue, will combine with the aldehydes and prevent their action on the cyanide. De Jong,[16] in working with the decomposition of gynocardin in the leaves of *Pangium edule*, found that a diketone and HCN were produced, which combined in part, thus holding back some of the prussic acid from distillation.

It is also evident from Table II that grinding with tartaric acid completely inhibits enzyme action, as in two cases, Nos. 88a and 91a, no cyanide was obtained in this way, whereas by normal hydrolysis from 5 to 7 mg. were obtained. In No. 87, where cyanide was obtained even with the acid treatment, this cyanide no doubt existed in some non-glucosidic form. This will be discussed under another heading.

[16] De Jong, A. W. K., La décomposition de la gynocardine par l'enzyme des feuilles de pangium edule, *Rec. trav. chim. Pays-Bas*, 1911, xxx, 220.

Effect of Vacuum Distillation on the Yield of Cyanide.

In order to ascertain whether distillation under reduced pressure, and hence lower temperature, would decrease the retention of cyanide, the experiments in Table III were carried out. After removing the cyanide under reduced pressure, redistillation was found to be necessary because foaming had discolored the distillate. It is unlikely that this redistillation had any effect on the cyanide. In two of the cases a slight increase in hydrocyanic acid in favor of vacuum distillation is seen, but it is inappreciable.

TABLE III.

The Effect of Distilling Macerated Sample 1½ Hrs. at 50-55°, 15 Mm. Pressure, Then Redistilling at Ordinary Pressure.

Nos.	Description of samples.	HCN obtained.	
		By ordinary distillation.	By vacuum distillation.
		mg.	*mg.*
72a and 72b	Ground with 5 per cent tartaric.	0.40	0.40
76a " 76b	" " 5 " " "	0.00	0.20
82 " 83	" " 5 " " " and 1.0 mg. HCN (as KCN) added.	0.60	0.80

Form of the Occurrence of Hydrocyanic Acid in Sorghum.

In the discussion of the data in Table II, it was pointed out that grinding in the presence of tartaric acid completely prevented enzyme action on glucoside. Nevertheless, cyanide was obtained in this way in some cases. Assuming that the acid cannot appreciably affect dhurrin, this cyanide must have existed in the plant in some non-glucosidic form. The only reference the writer could find on the nature of the hydrocyanic acid compounds of sorghum was one by Dunstan and Henry,[17] who, in discussing their newly found sorghum glucoside dhurrin, said that the hydrocyanic acid apparently does not occur in the free state, but only as the glucoside. They did not state their reasons for this conclusion. In Table IV is brought together the evidence which

[17] Dunstan, W. R., and Henry, T. A., Cyanogenesis in plants. II. The great millet, *Sorghum vulgare*, *Chem. News*, 1902, lxxxv, 301.

points towards the existence in *Sorghum vulgare* of non-glucosidic hydrocyanic acid. It all hinges upon two assumptions: first, that the method of grinding with 5 per cent tartaric acid prevents any

TABLE IV.

The Occurrence of Glucosidic and Non-Glucosidic Hydrocyanic Acid in Sorghum.

Nos.	HCN per 100 gm. of dry weight of leaves.		
60, 61, 62	I. Leaves ground, then distilled from 5 per cent tartaric, allowing about 5 min. autolysis. 7.12 mg.	II. Leaves ground and distilled from water, allowing about 8 min. autolysis. 7.12 mg.	III. Autolyzed 24 hrs. at 45°, then distilled from tartaric. 12.25 mg.
69, 70, 65	I. Ground with tartaric, all enzyme action prohibited. 5.13 mg.	II. Ground and distilled from water, about 8 min. autolysis. 12.40 mg.	III. Autolyzed 24 hrs. at 45°. 12.40 mg.
76a, 87, 84	I. Ground with tartaric, all enzyme action prohibited. 0.00 mg.	II. Same sample as I, but frosted, ground with tartaric, all enzyme action prohibited. 1.74 mg.	III. Same sample as II, ground and distilled from water, about 8 min. autolysis. 10.45 mg.
89a1 and 82a2	I. Ground with tartaric, all enzyme action prohibited. 0.00 mg.	II. Ground and autolyzed 4 hrs. at 23°. 4.50 mg.	
90a1 and 90a2	I. Ground with tartaric, all enzyme action prohibited. 4.50 mg.	II. Ground and autolyzed 4 hrs. at 23°. 11.25 mg.	

enzyme decomposition of glucoside; and second, that the acid itself does not accomplish any hydrolysis even during boiling. The first is based upon the fact that a given sample of sorghum

leaves will yield cyanide after autolysis, but will not yield any if ground in presence of tartaric acid; and that still other samples give some cyanide when ground with the acid, but will give more if not ground with it. The second assumption is based on the following facts: (1) Amygdalin cannot be hydrolyzed with 5 per cent tartaric acid (see Table I) and amygdalin and dhurrin are very similar in their composition and properties. (2) Samples of leaves which yield hydrocyanic acid by autolysis, yield none on boiling with tartaric acid. Therefore it is believed that sorghum does not contain all of its hydrocyanic acid in the form of dhurrin; that at times part or all of it may exist in some more unstable compound which can be decomposed by boiling 5 per cent tartaric acid. Whether the prussic acid actually exists free in certain of the cells, as found by Treub in *Pangium edule*, or even as the salt of metals, the data at present do not show. Peche[3] suggests that in *Prunus laurocerasus* it may be linked with a ketone. Treub,[1] in demonstrating the presence of free hydrocyanic acid in *Pangium edule*, treated the leaves with boiling alcohol to prevent enzyme action. De Jong[18] modified this by using large volumes of absolute alcohol at $-10°C$.

This discovery may explain the apparent discrepancy between Auld's[15, 19] results from the study of the possibility of cyanide poisoning in stock, and what actually happens in practice. He concluded, after a study of the conditions favorable for the liberation of cyanide in feedingstuffs by enzymes, that such hydrolysis is unlikely except under unusual circumstances, since it is prevented by the reaction of the alimentary pouches, and by the presence of large amounts of fiber, salt, glucose, etc. Cyanide poisoning, however, is very common in districts growing sorghum, and it is probable that it is caused in large part by the non-glucosidic hydrocyanic acid.

The demonstration of the inefficiency of tartaric acid as a hydrolyzing agent for dhurrin, of the rapidity of autolysis on grinding the tissues, and of the existence of non-glucosidic hydrocyanic acid in sorghum, necessitates a revision of the conclusions arrived

[18] De Jong, L'acide cyanhydrique des feuilles du *Pangium edule*, *Ann. Jardin Bot. Buitenzorg*, 1908, xxii, 1.

[19] Auld, Cyanogenesis under digestive conditions. *J. Agric. Sc.*, 1913, v, 409.

at by Willaman and West[12] in regard to the effect of climatic factors on the prussic acid content of sorghum. The method employed there throughout the season was to grind the tissues, and then put them into 5 per cent tartaric acid. From the evidence given above, this allowed from 4 to 7 minutes for autolysis; a time sufficient for considerable, if not for complete, hydrolysis. If it was complete, or if the degree of hydrolysis was the same in all cases, the comparative value of the data would still obtain. If there was considerable variation in the relative amount of the total cyanide in the non-glucosidic condition, there would also be a corresponding variation in the amount of hydrolysis of the glucoside, and the data would carry but little significance. Which of these two cases is correct remains for future work to determine.

It would be of interest to study the form of occurrence of the prussic acid in the hosts of cyanogenetic plants known to science. No doubt more exact information would be obtained as to the nature of this non-glucosidic union of cyanide, as well as to the variation in the proportion of glucosidic to non-glucosidic cyanide present under various conditions.

SUMMARY.

1. The methods in use for the determination of the hydrocyanic acid content of plant tissues are of questionable accuracy, because of the difficulty in getting complete hydrolysis of the glucosides by means of acids, and because of the retention of the cyanide from distillation by the tissues involved.

2. Hydrolysis of the dhurrin in sorghum is best accomplished by means of the glucosidase found in the same tissues (autolysis). It takes place very rapidly at 45°C.

3. Retention of hydrocyanic acid by the tissues during distillation cannot be prevented by the presence of tartaric acid, nor can it appreciably be lessened by distilling under reduced pressure.

4. *Sorghum vulgare* contains hydrocyanic acid in two forms; a glucosidic, as dhurrin, and a non-glucosidic, the nature of which is as yet unknown. It is the latter portion of the cyanide of this plant which is probably responsible for the poisoning of stock.

5. The non-glucosidic cyanide can be distinguished from the glucosidic by grinding the leaves in the presence of 5 per cent tartaric acid to prevent any enzyme action, and then distilling.

THE EFFECT OF ANESTHETICS AND OF FROSTING ON THE CYANOGENETIC COMPOUNDS OF SORGHUM VULGARE.

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INTRODUCTION.

Some interesting phenomena have been observed arising from the effect of anesthesia on living plants. The forced premature flowering of certain plants by means of ether is a commercial practice,[1] especially applicable to plants in a dormant condition, as bulbs and twigs in the late winter. It is believed, in general, that the entrance of the anesthetic increases the permeability of the tissues, with a consequent more rapid absorption of water. Some anesthesia phenomena are thus explained by the bringing together of substrates and enzymes.

The most carefully worked out explanation of the effect of anesthetics is contained in the work of the Armstrongs.[2, 3] The material for their study was the leaves of the cherry laurel, *Prunus laurocerasus*, containing the cyanogenetic glucoside prulaurasin. As an index of the effect of anesthetics they used Mirande's[4] test for the liberation of hydrocyanic acid from plant tissue. The test consists in immersing about two-thirds of the

[1] Duggar, B. M., Plant Physiology, New York, 1911, 335.

[2] Armstrong, H. E., and Armstrong, E. F., The origin of osmotic effects. III. The function of hormones in stimulating enzymic change in relation to narcosis and the phenomena of degenerative and regenerative change in living structures, *Proc. Roy. Soc., Series B*, 1909–10, lxxxii, 588.

[3] Armstrong and Armstrong, The function of hormones in regulating metabolism, *Ann. Bot.*, 1911, xxv, 507.

[4] Mirande, M., Influence exercée par certaines vapeurs sur la cyanogenèse végétale. Procédé rapide pour la recherche des plantes à acide cyanhydrique, *Compt. rend. Acad.*, 1909, cxlix, 140.

tissue, as a leaf, for example, in water containing the anesthetic. The latter enters the leaf and brings about the hydrolysis of the contained cyanogenetic glucoside and thus the liberation of hydrocyanic acid, which is transpired from the unimmersed portions of the leaf and produces a color change in moist sodium picrate paper suspended in the tube over the leaf. The time required for the appearance of the color change is a rough index of the intensity and rapidity of the action of the anesthetic. The Armstrongs employed a large number of substances in their attempt to explain the mechanism of the reaction. They found that the substances with the least attraction for water, or the anhydrophyllic, exerted the most marked effect, while the hydrophyllic had the least. Thus chloroform, carbon bisulfide, ether, and ammonia were strongly active; benzene, naphthalene, and thymol were moderately active; while most inorganic salts and weak acids were inactive.

From these results they concluded that anesthetizing substances possess their action by virtue of their lack of attraction for water; that their molecules can pass through the differential septa of plants without becoming involved in the aqueous structure thereof; that "such interposition, however, at once alters the osmotic state by promoting dissociative chemical change in the water in the direction $(H_2O)_x \rightarrow xH_2O$, ... and the equilibrium is disturbed, so that an influx of water from other regions sets in."

The reaction in plants brought about by anesthetics, then, is one of hydrolysis. It is not necessarily the hydrolysis of glucosides, however, for the disturbance in the tissues may bring together any substrate and its enzyme.

One other study similar to that of the Armstrongs should be mentioned. That is the work of Clark[5] on wild indigo. Anesthetics in this case cause the hydrolysis of a glucoside into an aromatic compound, which then is oxidized by an oxidase to a black pigment, causing the whole leaf to turn black. He found acetone and then the lower esters to be the most active in bringing about this effect.

[5] Clark, E. D., Note on the blackening of the leaves of the wild indigo (*Baptisia tinctoria*) and the isolation of a new phenol, baptisol, *J. Biol. Chem.*, 1915, xxi, 645.

In the investigations on cyanogenesis in the common sorghum made by the writer[6] in 1915, chloroform and alcoholic sodium hydroxide were used as a preservative in shipping the plants in cans from various field plots to the laboratory for analysis. As a control measure, samples from the home plots were analyzed before and after preservation, at all stages of growth, to detect any unwonted effect of the preservation in the hydrocyanic acid content. As shown in Table I in the report of those experiments, no effect was noticed through those stages of growth that were involved in the experiments. As the plants become older, however, it became increasingly evident that more cyanide was obtainable after several days' preservation in chloroform than before. A number of experiments were tried, varying different factors, and involving plants of different ages. The plant parts were cut into 1 inch lengths, and 100 gm. were placed in a 500 cc. tin can with a closely fitting cover. The preservative was added and the material allowed to stand at room temperature the specified length of time. The method of analysis was to grind the tissue in a food chopper, add immediately to 5 per cent tartaric acid, and distill slowly into a little sodium hydrate solution until a volume of 100 cc. was obtained. The hydrocyanic acid was estimated colorimetrically by the method of Viehoever and Johns.[7] As was discussed in a previous paper,[8] this tartaric acid distillation does not liberate all the hydrocyanic acid in the tissues; but since all the samples in these experiments received almost identical treatment, the results are comparable one with the other.

Some of the data obtained concerning the effect of the anesthetics are reproduced in Table I. Chloroform, alcohol, and ether, when acting on the older plants, apparently cause an increased yield of prussic acid; this effect is very marked in some cases. The presence of sodium hydrate in the alcohol decreases

[6] Willaman, J. J., and West, R. M., Effect of climatic factors on the hydrocyanic acid content of sorghum, *J. Agric. Research*, 1916, vi, 261.

[7] Viehoever, A., and Johns, C. O., On the determination of small quantities of hydrocyanic acid, *J. Am. Chem. Soc.*, 1915, xxxvii, 601.

[8] Willaman, J. J., The estimation of hydrocyanic acid and the probable form in which it occurs in *Sorghum vulgare*, *J. Biol. Chem.*, 1917, xxix, 25.

TABLE I.

The Effect of Various Anesthetic Preservatives on the Hydrocyanic Acid Content of Sorghum.

No.	Method of preservation.	Portion of plant used.	Height of plant.	HCN of dry matter.	
				Without preservation.	With preservation.
			ft.	*per cent*	*per cent*
P 16	20 cc. alcoholic NaOH + 4 cc. $CHCl_3$, 4 days.	Whole plant.	2.4	0.019	0.020
P 18	20 " " " 4 " " 4 "	Whole plant.	2.4	0.026	0.029
P 27	20 " " " 4 " " 8 "	Whole plant.	3.3	0.009	0.009
P 29	20 " " " 4 " " 8 "	Whole plant.	3.3	0.016	0.019
P 32	30 " " " 4 " " 3 "	Leaves.	4.5	0.004	0.014
P 34	30 " " " 4 " " 3 "	"	4.5	0.007	0.031
P 35a	30 " " " 4 " " 3 "	"	5.7	0.004	0.020
P 35b	30 " " " 4 " " 6 "	"	5.7	0.004	0.030
P 21	20 " alcohol, 4 days.....	"	6.5	0.001	0.022
P 22	20 " " + 2 cc. $CHCl_3$, 4 days.....	"	6.5	0.001	0.017
P 23	20 " " + 2 " ether, 4 "	"	6.5	0.001	0.017
P 24	20 " 2 per cent alcoholic NaOH.....	"	6.5	0.001	0.009
P 38	No preservative, 2.5 days.....	"	6.8	Trace	Trace
P 39	30 cc. alcohol, 2.5 days.....	"	6.8	"	0.012
P 43	30 " " + 2 cc. $CHCl_3$, ½ hr.....	"	6.8	"	0.001
P 44	30 " " 2 " " 2.5 days...	"	6.8	"	0.004
P 46	20 cc. alcoholic NaOH + 2 cc. $CHCl_3$, ½ hr.	"	6.8	0.003	0.010
P 47	20 " " " 2 " " ½ "	"	6.8	0.003	0.010

the effect somewhat, probably because of the hardening of the epidermis and the consequent lesser penetration of the alcohol.

Three possible explanations of this phenomenon are suggested: (1) the disturbed osmotic relations in the tissues bring about increased hydrolysis of the cyanogenetic glucoside dhurrin; (2) the presence of the anesthetics stimulates not only the hydrolytic

activities of the enzymes, but their synthetic activities as well, resulting in this case in an actual production of more dhurrin and more hydrocyanic acid; (3) a combination of both (2) and (1).

EXPERIMENTAL.

In order to obtain some further information on this subject, it was decided to carry on a few more experiments on the 1916 crop of sorghum. The plan was to subject a quantity of leaves to the action of chloroform vapor and then to extract and isolate the dhurrin itself, if possible, comparing the yield with that from the same quantity of untreated leaves. The method followed was that of Dunstan and Henry.[9] The leaves were dried in a current of air at about 45°C., ground to a fine powder, extracted twice with 95 per cent alcohol at the rate of 400 cc. of alcohol for each 100 gm. of leaf powder, the alcohol was evaporated, the residue taken up with water, freed from tannates by lead acetate, made into a paste with animal charcoal, dried, extracted in a Soxhlet for 72 hours with anhydrous ethyl acetate, and the extract slowly evaporated to crystallize out the dhurrin. The attempt to obtain crystalline dhurrin, however, resulted in a failure. In the first extraction dhurrin was present, but it was accompanied by too much coloring matter to be recovered in crystalline form. When it was decolorized by a second treatment with charcoal, crystals again failed to form. At each step of the process, however, an analysis was made for hydrocyanic acid, thus enabling one to follow the comparative cyanide content without obtaining the glucoside itself. In some cases the hydrocyanic acid was obtained by autolysis; in others by hydrolysis with emulsin.

The results of the experiments are given in Table II. It will be seen that in every case there is more hydrocyanic acid in the chloroformed than in the normal leaves, as shown by the analyses at each stage of the process. The analysis at each stage, however, has a different significance. In the first pair of samples, the first column in the table shows the prussic acid obtained by about

[9] Dunstan, W. R., and Henry, T. A., Cyanogenesis in plants. II. The great millet, *Sorghum vulgare*, *Phil. Tr. Roy. Soc., Series A*, 1902, cxcix, 399.

TABLE II.

The Effect of Chloroform Vapors on the Cyanogenetic Compounds of Sorghum.

No.	Treatment.	HCN per 100 gm. of dry weight of leaves. I. Original leaves ground, distilled from tartaric acid.	II. Leaves after treatment, ground and autolyzed 24 hrs. at 45°.	III. Leaves after treatment, dried, ground, and autolyzed 24 hrs. at 45° with emulsin.	IV. Dried and ground leaves extracted with alcohol and hydrolyzed with emulsin.
		mg.	*mg.*	*mg.*	*mg.*
52c	300 gm. of leaves packed in can 3 days at room temperature.	0.60			1.15
52d	300 gm. of leaves packed in can 3 days at room temperature with 9 cc. $CHCl_3$.	0.60			1.40
74	600 gm. of leaves same as 52c.	0.40	0.00	1.60	2.80
75	600 gm. of leaves same as 52d, but 18 cc. $CHCl_3$.	0.40	0.00	2.80	3.60

		I. Original leaves ground and autolyzed 4 hrs. at 23°.	II. Leaves after treatment ground and autolyzed 4 hrs. at 45°.	III. Leaves after treatment dried, ground, extracted with alcohol, and hydrolyzed with emulsin at 47° for 3 hrs.	
89	1,800 gm. of leaves packed in can 4 days.	5.51	4.50	0.26	
90	1,800 gm. of leaves packed in can 4 days with 53 cc. $CHCl_3$.	5.51	11.25	2.00	

8 minutes' autolysis previous to the addition of the tartaric acid. and includes, probably, all the non-glucosidic cyanide and part of the glucosidic, except that retained from distillation by the chemical action of the tissues.[10] The cyanide represented in the fourth column can have arisen only from dhurrin, since the drying of the leaves and the evaporation of the alcohol would have entirely removed any volatile cyanide before the hydrolysis by emul-

[10] See previous paper[8] for a discussion of this.

sin was performed. The difference in the yield of hydrocyanic acid here is a difference brought about by the *increased production of dhurrin in the leaves under the influence of the anesthetic.* The same can be said of the next pair of samples, Nos. 74 and 75. The third column shows an increased amount of dhurrin present in the leaves, as obtained by autolysis of the dried and powdered leaves; the fourth column shows an increased amount of dhurrin as obtained by extraction with alcohol. In the second column the prolonged autolysis caused the retention of all of the cyanide. In Nos. 89 and 90, the cyanide liberated by 4 hours' autolysis (second column) was doubled in the chloroformed plants. The glucosidic cyanide, extractable by alcohol (third column), decreased markedly in both, but much less in the treated than in the normal leaves. This shows *increased hydrolysis of the glucoside under the influence of the anesthetic,* and at the same time an *augmented synthesis of hydrocyanic acid.*

Evidently, then, the third proposition enumerated above holds true here. That is, when sorghum leaves undergo anesthesia by chloroform, both the synthetic and the hydrolytic activities of glucoside enzymes are increased. This in effect amounts to a case of demonstrated enzyme synthesis *in vivo.* It does not prove that the same enzyme is responsible for both the hydrolytic and the synthetic action towards dhurrin; in fact it rather proves the reverse, and substantiates Armstrong's hypothesis[11] that different enzymes are involved in the building up and the breaking down of a given substance. Since it is fair to assume that the chloroform affects primarily the enzymic activities of the tissues, then any increased production of glucoside must be due to synthetic enzyme activity.

In order to demonstrate, if possible, that an enzyme preparation from chloroformed leaves would reveal increased hydrolytic activity, enzyme powders were prepared from the residues of Nos. 89 and 90 (Table II) after extraction of the material with alcohol. The residues, freed from alcohol, were extracted with 2,000 cc. of water for 2 hours, filtered, 800 cc. of the filtrate were concentrated to 80 cc. at 30°C. under reduced pressure, poured into 600 cc. of 95

[11] Armstrong, E. F., Studies on enzyme action. III. The influence of the products of change on the rate of change conditioned by sucroclastic enzymes, *Proc. Roy. Soc.*, 1904, lxxiii, 516.

per cent alcohol, filtered immediately, and the precipitate dried. About 12 gm. of powder were obtained from each lot of material. The activities of the powders towards amygdalin were then determined by allowing 5 gm. of each to act on 0.3 gm. of amygdalin in 200 cc. of water at 47°C. for 2 hours. No. 89, from leaves not chloroformed, liberated 0.20 mg. of HCN; No. 90, from chloroformed leaves, liberated 5.00 mg. of HCN. Thus the chloroformed leaves yielded an enzyme powder about twenty-five times as active towards amygdalin as the powder from the same quantity of untreated leaves.

TABLE III.

The Effect of Frost on the Cyanogenetic Compounds of Sorghum.

Nos.	HCN per 100 gm. of dry matter.			
	Before frosting.		After frosting.	
	Non-glucosidic HCN.*	Glucosidic HCN.†	Non-glucosidic HCN.	Glucosidic HCN.
	mg.	*mg.*	*mg.*	*mg.*
76a and 87	0.00		1.74	
88 " 91	0.00	5.51	0.00	7.16

* Determined by grinding leaves with 5 per cent tartaric acid, thus preventing any enzyme action.

† Determined by grinding, then autolyzing 4 hrs. at 23°.

Sorghum has the reputation of being especially poisonous after it has been frosted. The question arose as to whether this increased toxicity was not due to a set of circumstances similar to those associated with anesthesia. To prove the point, a sample of frosted sorghum leaves (No. 87, Table III) was obtained on September 18, and analyzed for non-glucosidic hydrocyanic acid. Leaves from these same plants (No. 76a) on September 13, before being frosted, had already been analyzed. The results are given in Table III. In this case the frosting of the leaves had caused *profound hydrolytic changes.* Next, a sample of unfrosted leaves was obtained, and exposed in a refrigerating plant for $1\frac{1}{4}$ hours to a temperature of -7°C. After removal from here they were allowed to stand at 20°C. for 20 hours, and then analyzed, with the results shown in the table (Nos. 88 and 91). In this case no non-glucosidic hydrocyanic acid was obtained either before or af-

ter frosting, showing that no hydrolytic activity had been instigated by the low temperature. There was, however, a considerable increase in glucoside content, as obtained by 4 hour autolysis. Thus the low temperature had caused the *production within the tissues of more cyanogenetic glucoside* than existed before the frosting.

Although furnishing but meager evidence, these two examples seem to show that the effects of anesthetics and of frost are very similar. The rupturing of cells by the frost is no doubt the primary cause for changed osmotic conditions and increased permeability, although, as in the case of other plants, lower temperatures may allow certain enzymes to gain the ascendency over those normally in control at other temperatures.

SUMMARY.

1. Sorghum leaves exposed to the vapors of chloroform, ether, and alcohol, yield more hydrocyanic acid, both glucosidic and non-glucosidic, than the normal leaves.

2. The anesthetics thus stimulate both the hydrolytic and the synthetic action of the glucoside enzymes.

3. This is submitted as a case of demonstrated enzyme synthesis *in vivo*.

4. Enzyme powder prepared from chloroformed leaves was about twenty-five times as active towards amygdalin as the powder from untreated leaves.

5. Frosting also causes an increased yield of both glucosidic and non-glucosidic hydrocyanic acid. This is partly due to a rupturing of the cells, and partly to disturbed enzyme equilibrium.

FACTORS INVOLVING THE ACCURACY OF CREATININE DETERMINATIONS IN HUMAN BLOOD.

By ALEXANDER O. GETTLER.

With the Cooperation of Ruth Oppenheimer.

(From the Laboratory of Pathological Chemistry, Department of Pathology, Bellevue Hospital, New York.)

(Received for publication, December 5, 1916.)

Considerable discussion has arisen in various laboratories, since the publication, by Gettler and Baker,[1] of the micro analysis of normal blood, as to what should be considered the true normal value for creatinine. Shall it be 0.1 to 0.5 mg. in 100 cc. of blood, as given by the above authors, or shall the values of Folin and Denis,[2] which are many times higher, be adhered to?

McCrudden and Sargent[3] have since published the statement: "In the light of these [McCrudden and Sargent's] experiments, it is clear that all that has been written hitherto concerning . . . . creatinine and creatine in the blood will have to be rejected altogether." They arrive at this conclusion as a result of an observation that a color similar to that obtained by creatinine on picric acid in the presence of sodium hydroxide is produced by the sodium hydroxide on picric acid alone. They furthermore give experimental proof that five-sixths of the total color produced in the method is due to the alkali, and only one-sixth to the creatinine.

Had these authors read our publication more closely, they probably could have used our creatinine figures to bear out part of their contention, and at the same time modify their statement with an exception. In making their sweeping statement, however, I must here point out that the creatinine values published by Gettler and Baker do not warrant their severe criticism. The sources of error which they mention, and many others which

[1] Gettler, A. O., and Baker, W., *J. Biol. Chem.*, 1916, xxv, 211.

[2] Folin, O., and Denis, W., *J. Biol. Chem.*, 1914, xvii, 487.

[3] McCrudden, F. H., and Sargent, C. S., *J. Biol. Chem.*, 1916, xxvi, 527.

will be discussed in the experimental part, were recognized by us when we first started to use this method; they were quantitatively taken account of in all our work. That is why I now claim, after much experimental evidence in the laboratory, that the values of Gettler and Baker[1] are the correct ones, in preference to those of Folin and Denis.[2] It was Folin himself who called attention to the same effect of sodium hydroxide on picric acid. He even states that we can only then read our colorimeter with correct results, if the unknown color and the standard color are within one and a half times of each other.

In trying out this method previous to the determination of the creatinine values in normal blood,[1] we found at that time that Folin's suggestion was valid, but only for high values of creatinine (2 mg. in 100 cc. and over); when, however, it was a matter of estimating 0.1 to 0.5 mg. of creatinine in 100 cc. of blood, the above requirement was not sufficient. It was indeed necessary to have the standard and the unknown of the same depth of color, if we expected to get anywhere near the right value. To accomplish this in the simplest manner, we made up a series of standard creatinine solutions in picric acid, ranging from 0.1 to 2.1 mg. in 100 cc., as shown below. Each of these solutions we ran in the usual manner, adding oxalate and glucose in amounts as usually found in blood samples, and compared the colors in a Duboscq colorimeter with a M/72 dichromate solution. In this way we obtained a series of dichromate values, which were charted as follows:

Creatinine in 100 cc.	Placed at	M/72 bichromate reading.
mg.		
0.1	30	9
0.3	30	12
0.5	30	15
0.7	30	18
0.9	30	20
1.1	30	23
1.3	30	25
1.5	30	28
1.7	30	30
1.9	30	34
2.1	30	38

If now we compare an unknown (placed at 30) with this dichromate, and we obtain a reading of 15, the color of this reading is absolutely the same as given by 0.5 mg. of creatinine in 100 cc. It is evident that the unknown has thus been compared, indirectly, with a creatinine solution of the same strength.

The normal values of creatinine in blood are given as follows:

Folin and Denis[2]	1.1–1.4 mg. in 100 cc.
Myers and Fine[1]	1.0–2.0 " " 100 "
Gettler and Baker[1]	0.1–0.5 " " 100 "

It will be noticed that the values of Gettler and Baker are much lower than those of other authors; I attributed this difference at that time to our use of standards that were of the same depth as the unknown; but I made no quantitative investigation to prove the cause of this difference, and therefore laid no special stress upon it.

McCrudden and Sargent in their paper state that only one-sixth of the color, or $16\frac{2}{3}$ per cent, is due to creatinine, and five-sixths, or $83\frac{1}{3}$ per cent, to the alkali. They have determined this on a 0.5 mg. in 100 cc. creatinine solution. As far as they go, I have in the present investigation, been able to confirm their finding. But they have not investigated this point far enough. That alone would be no cause of error in the determination; for the comparison is made under similar conditions, with a standard creatinine solution in picric acid, and with exactly the same amount of alkali. Hence that same amount of additional color, due to the alkali, is obtained in the unknown as well as in the standard, and thus they cancel each other. The real cause for error McCrudden and Sargent do not call attention to. As will be shown in the experimental part, the lower the creatinine content the larger the percentage of color due to the alkali; on the other hand, the greater the concentration of creatinine the lower the percentage of color due to the alkali. Thus:

Concentration of creatinine, in 100 cc.	Color due to creatinine.	Color due to alkali.
mg.	*per cent*	*per cent*
0.1	20	80
0.5	66	33
1.0	80	20
2.0	85	15

[1] Myers, V. C., and Fine, M. S., *Post-Graduate*, 1915, xxx, 39.

From this it is plain that if we compare a standard containing 1 mg. in 100 cc. with an unknown containing 0.1 mg., the 20 per cent of the color due to alkali will cancel 20 per cent of the 80 per cent color due to alkali in the unknown, and the difference, or 60 per cent of the color, still due to the alkali, will automatically add itself to the 20 per cent (real value) and give 80 per cent, a value four times too high. On the other hand, if we compare a standard and an unknown of the same color, say the 1 mg. value above, then the 20 per cent of alkali color in the standard will just cancel the 20 per cent of color due to alkali in the unknown, and the value of 80 per cent that is obtained is correct.

It is furthermore evident from the above table that the higher the creatinine value, the less does the percentage of color due to the alkali become, and hence the less the effect of this on the creatinine value obtained. Thus if we compare a 0.1 mg. solution with a 1.0 mg. standard the value obtained is three times (300 per cent) too high. While if we compare a 2.0 mg. solution with a 1.0 mg. standard the error is only about 5 per cent and this is not much more than the experimental error in reading the Duboscq colorimeter.

So, in general, for values above 2 mg. in 100 cc. that have been published by various other workers, the error is not sufficient to warrant their rejection, as McCrudden and Sargent suggest. For values less than 1 mg. in 100 cc. the error becomes appreciable, until at a 0.1 mg. concentration the values read are four to five times too high.

EXPERIMENTAL PART.

Since this article was sent for publication, Hunter[5] and also Folin[6] have shown that picric acid in solution on standing, exposed to light, deteriorates gradually, and there is then obtained a deep coloration on adding alkali, which pure picric acid does not give. Therefore, to assure those who are interested in creatinine determinations, I will state that in this work full account of any such deterioration has been taken. On treating 10 cc. of purest recrystallized saturated picric acid solution, used through-

[5] Hunter, A., and Campbell, W. R., *J. Biol. Chem.*, 1916–17, xxviii, 335.
[6] Folin, O., and Doisy, E. A., *J. Biol. Chem.*, 1916–17, xxviii, 349.

out this work, with 1 cc. of double normal NaOH, as in the method, and placing the same at 30 (Duboscq) the M/72 standard bichromate reads 7.5; using this same picric acid solution after standing in the laboratory from 3 to 4 months (not in direct sunlight) and treating in the same way, it read 8.0. I would therefore suggest that, as each stock solution of picric acid is made up, it be read as above; if the reading is between 7.5 and 8.0 it can be put in a brown glass bottle and used directly; if it reads more, purification must be resorted to.

Experiment I. Effect of Potassium Oxalate on Creatinine Determinations.

As the actual creatinine values were not desired here, but only to show the relative colors, a series of tubes containing increasing amounts of dichromate were set up, instead of using the Duboscq colorimeter. The first tube marked (0) was of the same color as picric acid alone. Each succeeding tube was made a little deeper in color by a common increment of 0.25 cc. of saturated dichromate solution. Thus the higher numbers represent deeper colors, the lower numbers represent less color.

With this series of colors, those obtained from varying amounts of creatinine, with varying amounts of oxalate were compared, with the results given in the following table.

5 cc. creatinine solution containing per 100 cc.	Saturated picric acid solution.	2 N NaOH per 10 cc.	Relative values found in presence of units of oxalate.				
			No oxalate.	Saturated oxalate.			
				0.1 cc.	0.5 cc.	1 cc.	2 cc.
mg.	*cc.*	*cc.*					
0.1	to 25	1	1	−1	0	0	0
0.25	" 25	1	3	2	0	0	0
0.5	" 25	1	7	5	0	0	0
1.0	" 25	1	19	11	6	5	4
2.0	" 25	1	30	18	11	9	10
3.0	" 25	1	+30	21	19	20	15
4.0	" 25	1	+30	27	24	25	21

Deduction.—Potassium oxalate has a strong bleaching effect on the picramic acid color, as seen in the tabulation. Passing from

the fourth column, the numbers in which represent the normal color, and going to the right, the numbers diminish with increasing amounts of oxalate, signifying that the picramic color is appreciably decreased by the presence of oxalate. For example, 0.5 cc. of saturated oxalate in 5 cc. of blood has entirely destroyed the picramic acid color produced by 0.5 mg. of creatinine. Even 0.1 cc. of oxalate in 5 cc. of blood (0.5 cc. in 30 cc.) produces a noticeable decrease in color.

Hence in adding potassium oxalate to blood in order to prevent clotting, it is very essential either to weigh or measure the same. 1 cc. of a saturated solution to every 60 cc. of blood is sufficient (an amount as added to standards when making the table).

Experiment II. Effect of Glucose, in Concentration as Found in Normal Blood, Also That of Standing after the Addition of the Alkali.

Creatinine per 100 cc. actually present.	Glucose.	Saturated potassium oxalate solution.	Saturated picric acid solution.	2 N NaOH per 10 cc.	Colorimetric readings.		Creatinine per 100 cc.	
					10 min. after addition of NaOH.	30 min. after addition of NaOH.	From colorimetric readings.	
							After 10 min.	After 30 min.
mg.	*mg.*	*cc.*	*cc.*	*cc.*			*mg.*	*mg.*
0.1	+6	0.1	to 15	1	9.5	13.9	0.15	0.45
0.1		0.1	" 15	1	8.7	8.5	0.1	0.1
0.7	+6	0.1	" 15	1	20.7	38.7	0.95	2.1
0.7		0.1	" 15	1	17.4	17.4	0.68	0.68
2.0	+6	0.1	" 15	1	41.4		2.3	
2.0		0.1	" 15	1	34.3	34.3	1.9	1.9

Deduction.—Using the dichromate table, the values for creatinine obtained are the same as the amount of creatinine added. The presence of glucose (as in blood) gives slightly higher values. On standing longer than 10 minutes, the color developed by the glucose becomes appreciable. Hence, in making standards for comparison, glucose should be added in amounts as found in blood. Readings must be made after 10 minutes; delay introduces an element of error.

Experiment III. Effect of Alkali on the Values Obtained for Different Concentrations of Creatinine.

Creatinine per 100 cc. used.	Oxalate and alkali.	Colorimetric reading (Duboscq.)		Color due to each component.					
				In ratio.			In percentage.		
		Saturated picric.	Reading of other tube.	Picric.	Alkali and oxalate.	Creatinine.	Picric.	Alkali and oxalate.	Creatinine.
mg.							*per cent*	*per cent*	*per cent*
0	0	30	30	1	0	0			
0	*	30	17.7	1	0.7	0			
0.1	*	30	13.3	1	0.7	0.5	46	32	23
0.5	*	30	8.2	1	0.7	1.9	28	19	53
1.0	*	30	4.3	1	0.7	5.2	14	10	76
2.0	*	30	3.2	1	0.7	7.7	10	7	83

* Amounts as required by the method.

Deduction.—For values around 0.1 mg. in 100 cc. the color due to creatinine is only about 20 per cent of the total color produced. As the concentration increases, the percentage of color due to creatinine increases, at a 2.0 mg. concentration; it is 83 per cent of the total color, only 17 per cent is due to the picric and alkali. With still higher concentration, of course, this percentage of color due to alkali picrate becomes less and less.

Experiment IV. Derivation of Table to Be Used in the Colorimetric Determination of Creatinine in Blood.

Creatinine per 100 cc. (Used 3 cc.)	Saturated picric acid solution containing 100 mg. of glucose in 100 cc.	Saturated potassium oxalate solution.	2 N NaOH per 10 cc.	Colorimetric readings after 10 min.	
				Standard M/72 bichromate.	Standard creatinine.*
mg.	*cc.*	*cc.*	*cc.*		
0.1	to 15	0.1	1	9	30
0.3	" 15	0.1	1	12	30
0.5	" 15	0.1	1	15	30
0.7	" 15	0.1	1	18	30
0.9	" 15	0.1	1	20	30
1.1	" 15	0.1	1	23	30
1.3	" 15	0.1	1	25	30
1.5	" 15	0.1	1	28	30
1.7	" 15	0.1	1	30	30
1.9	" 15	0.1	1	34	30
2.1	" 15	0.1	1	38	30

* Represents the unknown when using this table.

Experiment IV—Concluded.

Creatinine per 100 cc. (Used 3 cc.)	Saturated picric acid solution containing 100 mg. of glucose in 100 cc.	Saturated potassium oxalate solution.	2 N NaOH per 10 cc.	Colorimetric readings after 10 min.	
				Standard M/24 bichromate.	Standard creatinine.*
mg.	*cc.*	*cc.*	*cc.*		
2.1	to 15	0.1	1	14	30
2.5	" 15	0.1	1	16	30
3.0	" 15	0.1	1	19	30
3.5	" 15	0.1	1	22	30
4.0	" 15	0.1	1	27	30
4.5	" 15	0.1	1	31	30
4.5	" 15	0.1	1	19	20
5.0	" 15	0.1	1	22	20
6.0	" 15	0.1	1	29	20
7.0	" 15	0.1	1	34	20
8.0	" 15	0.1	1	40	20
9.0	" 15	0.1	1	46	20
10.0	" 15	0.1	1	51	20

How to Use the Above Table.—Provided Folin's method is adhered to, as in the above, the unknown is placed at 30, and then the reading obtained on the colorimeter is translated, into mg. of creatinine in 100 cc. of blood, directly by the figure in the first column.

Experiment V. Comparing the Values Obtained by Folin's Standard with Those of Our Table.

Creatinine per 100 cc. (Used 3 cc.)	Saturated picric acid solution containing 100 mg. of glucose in 100 cc.	Saturated oxalate.	2 N NaOH per 10 cc.	Creatinine found by Folin's standard.	Creatinine by Gettler's table.
mg.	*cc.*	*cc.*	*cc.*		
0.1	to 15	0.1	1	0.6	0.1
0.3	" 15	0.1	1	0.6	0.35
0.5	" 15	0.1	1	0.7	0.5
0.7	" 15	0.1	1	0.9	0.7
0.9	" 15	0.1	1	1.0	0.95
1.1	" 15	0.1	1	1.0	1.1
1.3	" 15	0.1	1	1.1	1.3
1.5	" 15	0.1	1	1.2	1.4
1.7	" 15	0.1	1	1.3	1.6
1.9	" 15	0.1	1	1.5	1.8
2.1	" 15	0.1	1	1.6	2.1

The above table shows plainly that for low values, as found in normal blood, the readings by the Folin standard are much too high. When the values reach 1 mg., as in the standard,[7] then only does the Folin standard give the correct value. Finally, if the unknown has a higher content than the standard, the values always run low. By means of the standardized table, however, the values obtained agree very closely with the amounts actually present.

Experiment VI. Some Additional Normal Creatinine Values.

No.	Name.	Reading of colorimeter.	Creatinine per 100 cc.
			mg.
1	Mc	10	0.15
2	H	9	0.1
3	D	8	0.1
4	Sh	8	0.1
5	K	9	0.1
6	G	13	0.4
7	T	12	0.3
8	J	9	0.1
9	R	10	0.15
10	W	11	0.2
11	E	8	0.1

Deduction.—The normal value for creatinine in blood is 0.1 to 0.5 mg. in 100 cc. This confirms the values of Gettler and Baker. The values of Folin and Denis[2] and of Myers and Fine[4] are much too high.

SUMMARY.

I. In the light of the experimental evidence just presented, I cannot subscribe to McCrudden and Sargent's view, that all creatinine blood values heretofore reported are useless, because of error in method. The values, in normal blood, of Gettler and Baker[1] are correct and are here again confirmed. Furthermore,

[7] Folin's standard is 0.2 mg. of creatinine in 100 cc. Of this 10 cc. are used and colorized without diluting. It must be remembered, however, that the blood sample is diluted fivefold, so that the standard, calculating on a similar dilution, represents 1 mg. in 100 cc.

in all other reported values, which lie at 2 mg. in 100 cc. and above the error is not enough to warrant rejection.

II. The method for estimating creatinine in blood has been critically studied as to the following: (*a*) Oxalate has a strong bleaching action on the picramic acid color, so that care must be taken not to add too much. (*b*) Standing longer than 10 minutes brings in a large error, because color changes rapidly thereafter. (*c*) The total color that is read in the colorimeter is not all due to creatinine. At 0.1 mg. concentration it is only about 23 per cent of the total. As the creatinine content increases the percentage of color due to it increases, so that at a 2.0 mg. concentration it is 83 per cent and beyond that it more and more approaches 100 per cent.

III. A table is given for reading mg. of creatinine in 100 cc. of blood directly, in which all the above sources of error have been accounted for.

IV. Known solutions of creatinine have been determined with the Folin standard and also with this table, conclusively proving that for values of 0.1 to 0.5 mg. of creatinine the Folin standard gives values much too high.

V. Eleven additional normals have been determined for their creatinine value. These range between 0.1 and 0.4 mg. in 100 cc., confirming Gettler and Baker's previous report.

The values of 1.0 to 2.0 mg. in 100 cc. as reported by Folin and Denis, and by Myers and Fine, are much too high.

THE BEHAVIOR OF CHICKENS FED RATIONS RESTRICTED TO THE CEREAL GRAINS.*

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Plate 1.

(Received for publication, December 18, 1916.)

With the prevailing interest in problems of animal nutrition some confusion will probably arise if investigators translate without reserve and without definite knowledge the results secured with laboratory animals to those of economic value or to man himself. In investigations carried on from this laboratory, involving rats, swine, and cattle, there has been constant agreement among these three species in the effects produced by the several rations studied.

There may have been some slight differences between the resistances of swine and rats to certain rations involved in our extended inquiries, but on the whole substantial agreement has resulted. It does not follow, however, that this would be true with every ration that may arise for investigation, nor should we expect the requirements for the normal factors of nutrition to be exactly identical among all species of mammals. For purposes of a definite record of the behavior of different species we desire to record here the fact that chickens behave entirely differently from either rats or swine on rations limited to the corn (maize) or wheat grains and their products.

With swine or rats[1] there is practically no growth when the ration is restricted to either a corn meal, gluten feed mixture—or a wheat meal, wheat gluten ration. On the corn meal, gluten feed ration swine will maintain themselves for a long time—at

* Published with the permission of the Director of the Agricultural Experiment Station.

[1] Hart, E. B., and McCollum, E. V., *J. Biol. Chem.*, 1914, xix, 373.

least several months—without evidences of serious malnutrition; ultimately, however, their coats will show some roughness, the appetite will wane, and slight stiffness may arise, but no serious pathological symptoms develop, at least in the periods under our observation, 6 months. However, there will be little if any growth and no reproduction. When a suitable salt mixture is added to the corn grain ration, growth at a fair rate and continued well-being result. Reproduction likewise occurs, although it will not be normal.

Where wheat, wheat gluten forms the ration pathological conditions will manifest themselves with swine or rats in 4 to 5 months. Maintenance fails and marked symptoms of nutritional derangement develop. In the presence of a salt mixture there may be some early growth, but after a few months' restriction to this ration loss in weight sets in accompanied by important histological changes in the spinal cord.[2]

With chickens, starting at 3 to 4 pounds' weight, no such results as described above for mammals were obtained, and our observations extended over periods of 7 months' duration and in some cases for 12 months. On either the corn, gluten feed, or wheat, wheat gluten rations, these animals will make slow growth, but what is more important, they will reach maturity, maintain themselves without pathological symptoms developing, and produce fertile eggs. With swine or rats there is complete cessation of the œstrum on rations limited to either of these two grains, fortified only with the protein concentrate from the same grain.

With the chickens whose records are here given there was in many cases a fall in weight at the time of the laying period, and not enough time elapsed between the termination of the laying period and the termination of the experimental observations to regain the maximum weight. There was, however, variation among the individuals in this respect and a considerable number showed no such fall in weight during the laying period. It is to be expected that under unfavorable environmental conditions, such as strict housing and limited quarters, variations such as these would occur. The same results, however, may be obtained

[2] Hart, E. B., Miller, W. S., and McCollum, E. V., *J. Biol. Chem.*, 1916, xxv, 239.

with chickens kept under normal conditions and fed standard rations.

To all the rations used in these studies there was an addition of calcium carbonate to provide for egg shell production. This was a modification in the ash mixture of the ration and might be considered a crucial factor in the slow growth, maintenance, and reproduction secured with this species; nevertheless we have added to a wheat, wheat gluten ration calcium lactate, which should have modified the ration in the same way as here modified, but with absolute failure where swine were involved in the inquiry.

We have numerous records of failure with mammals where wheat served as a large proportion of the diet and have attributed such failure to the inherent toxicity in the grain.[3] Such disaster will result even where the animals (swine) are placed on the ration after reaching a weight of 75 to 100 pounds. This fact should eliminate any criticism that the success with chickens—limited to a wheat grain, wheat gluten, calcium carbonate ration—was made possible by the choice of birds already somewhat passed the half grown mark. Such toxicity as occurs in the wheat grain had no apparent detrimental effect on chickens.

We know, of course, that it is impossible to place baby chicks upon such rations as here used and have a successful rearing. It is possible, however, to take this species at the half grown period and obtain slow growth and fertile egg production. At what age or weight they can be started on such rations and secure growth and egg production is yet to be determined.

Perhaps we should not wonder at such results. It is apparent from paleontological records that this species (chickens) occupies a lower position in the scale of animal evolution than mammals, and the quantitative requirements of the normal factors for nutrition, established for a mammal, may not be exactly like those required by a fowl. It is certain from the data here presented that its mineral requirements for slow growth or maintenance are not like those of either the rat or pig, especially under conditions of a poor adjustment in the ration of the other factors for normal

[3] Hart and McCollum, *J. Biol. Chem.*, 1914, xix, 373. McCollum, E. V., Simmonds, N., and Pitz, W., *ibid.*, 1916, xxv, 105. Hart, Miller, and McCollum, *ibid.*, 1916, xxv, 239.

nutrition. We have, however, seen pigs[2] grow well on a grain diet, fortified with a protein concentrate like oil meal *and an addition of but 1 per cent of the ration as meat scraps.* The latter, in the proportion used, could not possibly have modified the mineral content of the ration in any important degree, but without its presence failure resulted. By what action such a small percentage of meat could turn a ration from a failure into a success for growth in mammals needs specific investigation. The extreme exponents of the vitamine theory would find a ready explanation in the cry of "deficiency," but that is not the explanation. Substituting butter fat for the meat does not make the ration a success.

What the differences are between species in respect to the requirements of the unidentified dietary factors A and B cannot be definitely decided from these data. But it is true that the two grains, corn or wheat, provide chickens with enough of these substances at the half grown period for continued slow growth, maintenance, and production of fertile and hatchable eggs, if we take the records of maximum performance; but future work must determine what are the requirements in respect to these factors by baby chicks making for normal growth and reproduction. We are assuming that this species cannot synthesize these unidentified dietary factors, an assumption which is undoubtedly correct, especially in reference to the unidentified factor B.

Osborne and Mendel[4] have recently pointed out that the same relation holds for chickens as for rats in respect to their dependence for normal growth on the qualitative make-up of the proteins.

The chicken's tolerance for wheat, without an adjustment of the nutritive factors of this grain, as is necessary with swine or rats, is evidence of a difference in its metabolism as compared with that of the mammal—a fact also supported by the well known differences between their purine metabolism.

EXPERIMENTAL.

For both seasons of experimental work vigorous pullets (Rhode Island Reds) were selected. There were three in each lot, confined to wire cages with shavings as litter and scratch. They

[4] Osborne, T. B., and Mendel, L. B., *J. Biol. Chem.*, 1916, xxvi, 293.

were placed in the cages November 1 and the experimental observations terminated June 1, a period of 7 months' observation. New birds were selected for the 2nd year; distilled water was used for all lots with the exception of one of the controls where well water was used. Quartz grits were used in the hoppers. The salts were mixed intimately with the feeds whether fed as dry or wet mash. Calcium carbonate was used as the precipitated carbonate in the proportion of 3 pounds to 100 pounds of air-dried feed. The protein concentrate was used either as dry or wet mash, but mixed with some of the cereal grain. In addition, some of the whole grain was cracked and used as a scratch feed. The proportion of grain to concentrate as indicated in the table was, however, always kept constant over any given number of days of consumption. This was accomplished by weighing out the proportion given in the table and then seeing to it that each proportion was fed and consumed before a new batch of material was started.

The feeds used in the check lots consisted of three parts of corn, two parts of wheat, one part of oats as scratch, and one part each of bran, middlings, and corn as mash. These were supplied at the rate of two parts of scratch feed to one of mash. The check ration is by no means a very successful one for rapid growth and large production with chickens, but what it accomplished as contrasted with its effects on swine or rats is especially what we want to emphasize. It would lead to absolute disaster with swine or rats, but with chickens it gave moderate growth and reproduction. It should be kept in mind that this ration was of strictly vegetable origin derived wholly from cereal grains and that it maintained this species with production of fertile eggs. In fact during the short laying period of 2 months exhibited by these birds (March 1 to May 1) there was averaged an egg per fowl, every 3rd day.

In addition to our studies of the effects of restricted corn and wheat rations on the growth and egg-laying production of chickens we have extensive records where the rations were restricted to oat grain or to barley grain and their products. No detailed discussion of these records will be given at this time, but the statement can be made that this species could maintain itself and pro-

duce fertile eggs on rations limited to these two sources as well as on corn or wheat grain and their products.

During the 2nd year of observation the records of egg production represent those collected and not those produced. Most of the eggs were broken and in some cases eaten. These differences between the two records in the 2 years were largely due to the fact that a new attendant, unaccustomed to the work, was in charge the 2nd year. The size of the eggs produced in either year was normal and there was no appreciable variation among lots in their fertility. The hatching power—the percentage of live chickens produced from a given number of eggs—was a normal figure.

Addition to either a corn grain, corn gluten or wheat grain, wheat gluten ration of a salt mixture, which at least with corn makes possible the growth of swine, here made little if any improvement in the ration.

The addition of butter fat and salts or of butter fat, salts, and casein likewise gave little if any improvement over rations not carrying these additions. It might be contended that these were practically maintenance experiments, but a study of the data will show that most of the birds made considerable gains in weight—15 to 25 per cent of their initial weight and some even more than this. Birds 179 and 529—wheat, wheat gluten, calcium carbonate ration—made increases of 30 and 40 per cent respectively over their initial weight. Approximately similar increases were made on the corn, gluten feed, calcium carbonate ration.

All these rations gave low egg production; we are not contending that they cannot be improved, because as a matter of fact they actually are improved in practice by the addition of meat scraps, granulated milk, free range, etc., but that they accomplished anything as contrasted with their effect upon a mammal is the point we wish to emphasize.

Some of the birds pulled their feathers, but this occurred more or less in all lots. Figs. 1 to 7 show the condition of some of these animals at the termination of the experiment. There was absolutely no indication of polyneuritis among the birds on any of these rations. For comparison, see an earlier illustration of a pig on a wheat, wheat gluten, salt mixture ration.[5] This species

[5] Hart and McCollum, *J. Biol. Chem.*, 1914, xix, 373, Fig. 1.

TABLE 1.

Record of Results, 1914–15. Duration, November 1 to June 1. Laying Period March 1 to May 1.

Ration.	Bird No.	Weight.			Egg production.	Fertility.	Hatching power.
		Initial.	Maximum.	Final.			
		lbs.	*lbs.*	*lbs.*		*per cent*	*per cent*
Corn meal 70 lbs.	171	3.9	4.7	3.8	27	100	50
Gluten feed 30 lbs.	172	3.6	4.6	3.7			
$CaCO_3$ 3 lbs.	173	3.9	4.75	4.15			
Quartz.							
Corn meal 70 lbs.	174	3.9	5.25	5.25	36	90	60
Gluten feed 30 lbs.	175	3.2	3.9	2.35			
$CaCO_3$ 3 lbs.	176	4.0	4.8	3.45			
K_2HPO_4 323 gm.							
Ca lactate 513 gm.							
Quartz.							
Corn 95.5 lbs.	189	4.2	4.5	4.0	34	100	60
Casein 2.5 lbs.	190	3.1		2.15			
K_2HPO_4 323 gm.	191	3.5	3.8	3.35			
Ca lactate 513 gm.							
$CaCO_3$ 3 lbs.							
Quartz.							
Wheat meal 95.5 lbs.	177	3.8	4.3	4.0	27	100	80
Wheat gluten 2.5 lbs.	178	3.3	3.5	3.15			
$CaCO_3$ 3 lbs.	179	3.6	4.6	4.15			
Quartz.							
Wheat 95.5 lbs.	180	2.9		1.3	33	90	90
Wheat gluten 2.5 lbs.	181	3.4		1.85			
K_2HPO_4 323 gm.	182	3.7	4.5	4.35			
Ca lactate 513 gm.							
$CaCO_3$ 3 lbs.							
Quartz.							
Wheat 95.5 lbs.	183	3.7	4.3	2.55	35	100	80
Casein 2.5 lbs.	184	3.1	3.6	2.8			
K_2HPO_4 323 gm.	185	3.7	4.85	4.35			
Ca lactate 513 gm.							
$CaCO_3$ 3 lbs.							
Quartz.							

TABLE 1—*Concluded.*

Ration.	Bird No.	Weight.			Egg production.	Fertility.	Hatching power.
		Initial.	Maximum.	Final.			
		lbs.	*lbs.*	*lbs.*		*per cent*	*per cent*
Wheat 95.5 lbs.	186	3.6	4.65	4.35	32	80	80
Casein 2.5 lbs.	187	3.3	5.25	4.85			
Butter fat 2 lbs.	188	3.9	5.35	3.75			
K_2HPO_4 323 gm.							
Ca lactate 513 gm.							
$CaCO_3$ 3 lbs.							
Quartz.							
Check, variety grain ration.	192	3.2	4.6	4.05	69	100	90
	193	3.5	4.5	4.0			
Distilled water.	194	3.8	4.75	3.9			
$CaCO_3$ 3 lbs.							
Quartz.							
Check, variety grain ration.	195	3.5	4.25	3.7	61	80	60
	196	3.2	4.3	3.7			
Well water.	197	4.4	5.85	4.25			
$CaCO_3$ 3 lbs.							
Quartz.							

TABLE II.

Record of Results, 1915–16. Duration, November 1 to June 1. Laying Period, March 1 to May 1.

Ration.	Bird No.	Weight.			Eggs collected.*
		Initial.	Maximum.	Final.	
		lbs.	*lbs.*	*lbs.*	
Corn meal 70 lbs.	521	5.4	6.8	4.6	4
Gluten feed 30 lbs.	522	3.3	4.8	3.6	
$CaCO_3$ 3 lbs.	523	4.5	5.1	3.4	
Quartz.					
Corn meal 70 lbs.	524	3.7	5.7	4.5	5
Gluten feed 30 lbs.	525	4.7	5.8	4.0	
K_2HPO_4 323 gm.	526	3.3	4.9	2.7	
$CaCO_3$ 3 lbs.					
Ca lactate 513 gm.					
Quartz.					

* Eggs collected. Most of them were broken in the nest or eaten.

TABLE II—*Concluded.*

Ration.	Bird No.	Weight.			Eggs collected.*
		Initial.	Maximum.	Final.	
		lbs.	*lbs.*	*lbs.*	
Corn 95.5 lbs. Casein 2.5 lbs. $CaCO_3$ 3 lbs. K_2HPO_4 323 gm. Ca lactate 513 gm. Quartz.	539 540 541	4.0 3.3 3.8	5.6 4.8 4.8	4.0 3.6 4.3	1
Wheat meal 95.5 lbs. Wheat gluten 2.5 lbs. $CaCO_3$ 3 lbs. Quartz.	527 528 529	4.8 3.7 3.4	5.9 4.5 4.8	3.2 3.4 4.0	0
Wheat meal 95.5 lbs. Wheat gluten 2.5 lbs. $CaCO_3$ 3 lbs. K_2HPO_4 323 gm. Ca lactate 513 gm. Quartz.	530 531 532	4.5 4.7 3.6	5.6 4.8 4.8	4.3 4.0 3.1	1
Wheat 95.5 lbs. Casein 2.5 lbs. $CaCO_3$ 3 lbs. K_2HPO_4 323 gm. Ca lactate 513 gm. Quartz.	533 534 535	4.9 3.5 3.9	6.4 4.6 5.6	4.7 2.6 4.3	9
Wheat 95.5 lbs. Casein 2.5 lbs. Butter fat 2 lbs. $CaCO_3$ 3 lbs. K_2HPO_4 323 gm. Ca lactate 513 gm. Quartz.	536 537 538	5.1 3.4 3.1	7.0 5.3 4.2	4.0 4.0 3.0	5
Check, variety grain ration. Distilled water. $CaCO_3$ 3 lbs. Quartz.	542 543 544	4.3 3.6 3.3	5.7 5.8 5.0	4.8 4.4 3.7	8
Check, variety grain ration. Well water. $CaCO_3$ 3 lbs. Quartz.	545 546 547	3.5 3.9 3.6	5.0 6.2 5.3	4.7 4.9 4.2	0

* Eggs collected. Most of them were broken in the nest or eaten.

tolerates this ration for a few months, but ultimately will begin to lose weight and pass into a miserable condition.

SUMMARY.

1. Chickens started at half the normal weight can make slow growth, maintain themselves, and produce fertile eggs on rations limited to corn meal, gluten feed, and calcium carbonate, or wheat meal, wheat gluten, and calcium carbonate.

2. These results are in marked contrast to our records with swine or rats where these rations lead to loss of weight, cessation of œstrum, and with wheat to a condition resembling polyneuritis.

3. It is apparent that the mineral requirements at least, and possibly the requirements for the other normal nutritive factors are not the same for chickens as they are for mammals. Further, the chicken's ability to tolerate, without disaster and without modification of the ration, the toxic material of wheat speaks for a metabolism distinct from that of swine or rats.

4. Where half grown chickens were used there was no important improvement in the rates of growth or egg-laying capacity by supplementing the grains with either salts, casein, or butter fat, or a combination of the three, as contrasted with the results secured with the grain, grain protein concentrate, $CaCO_3$ ration. The protein level in all cases was approximately 12 per cent. On all of these rations the number of eggs produced, although fertile, was limited. This fact, at variance with the best results of practice where animal protein concentrates have proved of great value as supplements to cereal grains for a large egg production, would suggest that either the higher plane—20 to 25 per cent—of protein intake of superior quality secured in practice by the use of the animal protein concentrates (meat scraps, milk, etc.) explains these differences, or else the latter contribute certain factors necessary for large egg production which the cereal grain concentrates or casein fail to carry. The matter needs further investigation.

EXPLANATION OF PLATE 1.

FIG. 1. Condition of a fowl at the end of 7 months on a ration of 95.5 pounds wheat meal, 2.5 pounds wheat gluten, 3 pounds $CaCO_3$, quartz, and distilled water. This animal was in as good condition as were fowls receiving the wheat meal plus casein, butter fat, and a salt mixture (see Fig. 3). Contrast this figure with the condition of a mammal receiving a similar ration.[5] This ration is fatal to a mammal.

FIG. 2. After 7 months on a ration of 95.5 pounds wheat meal, 2.5 pounds casein, 3 pounds $CaCO_3$, 323 gm. K_2HPO_4, 513 gm. calcium lactate, quartz, and distilled water. The animal was in splendid condition.

FIG. 3. After 7 months on a ration of 95.5 pounds wheat meal, 2.5 pounds casein, 2 pounds butter fat, 3 pounds $CaCO_3$, 323 gm. K_2HPO_4, 513 gm. calcium lactate, quartz, and distilled water. Apparently normal condition, although some of the feathers had been pulled. This fowl was in no better condition than those receiving the wheat ration, but without casein, butter fat, or the salt mixture (see Fig. 1).

FIG. 4. Photographed at the end of 7 months on a ration derived wholly from seeds; scratch, 40 pounds corn, 40 pounds wheat, 20 pounds oats; mash, 33 pounds bran, 33 pounds middlings, 33 pounds corn meal, distilled water, and quartz. Fed two parts of scratch to one of mash. A normal fowl in every respect. $CaCO_3$ was not present in this ration. Such a ration would terminate growth in a mammal (pig or rat) and induce loss in weight and serious pathological changes.

FIG. 5. After 7 months on a ration of 70 pounds corn meal, 30 pounds gluten feed, 3 pounds $CaCO_3$, distilled water, and quartz. From all appearances this was a normal fowl.

FIG. 6. On the following ration for 7 months: 70 pounds corn meal, 30 pounds gluten feed, 3 pounds $CaCO_3$, 323 gm. K_2HPO_4, 513 gm. calcium lactate, distilled water, and quartz. Appeared in better condition than the individual shown in Figs. 5 or 7. These differences are really due to feather picking exhibited more by some individuals than by others.

FIG. 7. Photographed after 7 months on a ration of 95.5 pounds corn meal, 2.5 pounds casein, 3 pounds $CaCO_3$, 323 gm. K_2HPO_4, 513 gm. lactate, distilled water, and quartz. In good physical condition, but had pulled feathers.

FIG. 1

FIG. 2

FIG. 3

FIG. 4

FIG. 5

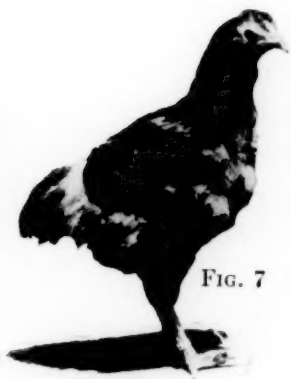

FIG. 7

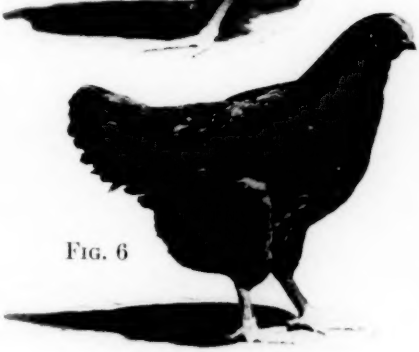

FIG. 6

THE RELATIVE VALUE OF CERTAIN PROTEINS AND PROTEIN CONCENTRATES AS SUPPLEMENTS TO CORN GLUTEN.*

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It has become more and more evident for some time that the question of the protein requirement, particularly in the case of farm animals, is in need of critical review if not of thorough revision. Practical experience has long since demonstrated that certain of the vegetable proteins, such as those of the maize kernel, fail to promote growth effectively unless they are supplemented by added nutrients. The deficiencies may be of more than one sort; but among them the inadequate or qualitatively inappropriate character of the protein is a clearly demonstrated feature. The evidence which has lately been offered for the dietary need of suitable amino-acids that cannot be synthesized by the animal organism has directed emphasis to the real significance, from the standpoint of the protein requirement, of supplementing one food by another containing a different variety of proteins.[1] It has become clearer that such inadequate but perhaps cheap proteins can be supplemented advantageously by one which supplies the needed factors, *i.e.*, amino-acids. Thus in attempting to improve a diet of which corn or corn meal forms the chief constituent our experiments have indicated that better results would be obtained if the added protein concentrate furnished

* The expenses of this investigation were shared by the Connecticut Agricultural Experiment Station and the Carnegie Institution of Washington, D. C.

[1] Mendel, L. B., Harvey Society Lectures for 1915–16; also *J. Am. Med. Assn.*, 1915, lxiv, 1539.

protein rich in tryptophane and lysine.[2] Hitherto animal fodders have been valued largely on the basis of the *amount* of protein which they contain, little attention being paid to the qualitative character of the protein. The principle that the quality of the protein should be considered in order to make sure that the ration is not deficient in any of the essential amino-acids is beginning to find expression in the most recent books on the feeding of farm animals,[3] but suitable directions for its application in practice remain to be formulated. Inasmuch as differences exist in the economy of correcting different inadequate protein foods with appropriate supplementary proteins, one problem involves the discovery of suitable readily available mixtures which shall not have any serious relative shortage of any essential amino-acid group.

Indications of the possibilities in this field of investigation have already been furnished by our earlier experiments with growing rats.[4] Thus experiments with additions of other proteins to corn gluten, containing a mixture of corn proteins with which only very slow growth is possible in rats even when all other (non-protein) essential dietary components are supplied, have shown results in accord with what one might expect from the presumable amino-acid make-up of the proteins added. Lactalbumin, rich in both tryptophane and lysine, appears to be a most efficient adjuvant; casein or edestin must be added in far larger proportion to accomplish results approaching those of the lactalbumin. In experiments to determine the relative values for milk production of different supplements to corn, Hart and Humphrey[5] found

[2] Osborne, T. B., and Mendel, L. B., *J. Biol. Chem.*, 1914, xvii, 325; 1914, xviii, 1; 1916, xxv, 1; 1916, xxvi, 293. See also Hogan, A. G., *ibid.*, 1916, xxvii, 193. The initial failure of Hogan to cause any improvement in the growth of rats by additions of lysine and tryptophane to his basal corn ration was presumably due to some deficiency in the diet other than the protein factor, making it impossible for the beneficial effect of the amino-acids to manifest itself. The effect of any specific addition to a ration can only be determined when all the other conditions are known to be satisfactory.

[3] Cf. Bull, S., The Principles of Feeding Farm Animals, New York, 1916, pp. 82, 131.

[4] Osborne and Mendel, *J. Biol. Chem.*, 1914, xviii, 1.

[5] Hart, E. B., and Humphrey, G. C., *J. Biol. Chem.*, 1915, xxi, 239; 1916, xxvi, 457.

that the milk proteins had a percentage efficiency of about 60, oil meal 61, distillers' grains 60, gluten feed 45, corn 40, and wheat 36.

To obtain a further survey of the possibilities of measuring the comparative nutritive potencies of different proteins or protein concentrates by the methods of feeding which we have pursued successfully for some years with rats and more recently with chickens,[6] we have undertaken a large number of additional experiments. Most of them involve the question of growth on diets made adequate in all details except the protein used. In this respect they differ from most earlier feeding trials with farm animals. In one series with a liberal supply of inadequate corn gluten as the basis of the nitrogenous part of the ration, diets were made with combinations containing varying proportions of different proteins, either isolated or in the form of protein fodder "concentrates," but in which the total amount of protein was substantially the same. These experiments soon showed that the increments of body weight may vary very widely with the *proportion* of any particular protein product added; or they may show variations related to the *same proportion of different proteins*, in an otherwise unchanged food mixture. For example, if rations containing a fixed proportion of total protein—about 16 per cent in the case of our standard foods—are prepared with increasing proportions of a protein like lactalbumin or casein and correspondingly decreasing amounts of the basal corn gluten, the effect is clearly reflected in the character of the growth which they afford. Occasionally, however, an animal will eat so much more of a food relatively inferior, from a chemical standpoint, that the *absolute* intake of the preferable protein will be comparatively large despite its relative paucity, *i.e.*, low percentage content, in the food mixture.

It was pointed out in an earlier paper[7] that no strict comparison can be made between the different proteins unless the total calorie intake, the absolute amount of protein eaten, the quantities of inorganic salts, and amount of "food accessories" are uniform. Strict comparisons of the nutritive value of proteins in

[6] Osborne and Mendel, *J. Biol. Chem.*, 1916, xxvi, 293.
[7] Osborne and Mendel, *J. Biol. Chem.*, 1916, xxvi, 1.

growth will therefore only be trustworthy when the experiments are comparable with respect to the total amount of such a food eaten during a definite period of time.

Experiments with carefully *limited* daily feeding in the manner described in the earlier paper are exceedingly laborious. We have therefore conducted many further trials in which the food mixtures containing unlike combinations of different proteins were offered *ad libitum* to rats. The proteins and protein concentrates used in these experiments are as follows:

1. Casein.—Prepared in this laboratory by precipitation from separator milk with hydrochloric acid, and subsequently purified by several reprecipitations and extractions with alcohol.

2. Lactalbumin.–Prepared in this laboratory by heat coagulation of the filtrate from the casein precipitate.

3. Edestin.—Prepared in this laboratory by extraction of ground hemp seed with sodium chloride and repeated solution and precipitation by dilution and cooling.

4. Cottonseed Protein.—Prepared in this laboratory by extraction of cottonseed flour with sodium hydroxide and precipitation by neutralization.

5. Corn Gluten.—Prepared for us by the Corn Products Refining Co.[4] Nitrogen content = 6.4 per cent (N × 6.25 = 40.0 per cent protein).

6. "Milkalbumin."—A commercial product containing 8.5 per cent of nitrogen (N × 6.38 = 54.2 per cent of protein) and 23.8 per cent of ash.

7. Cottonseed Flour.—From the Schulenburg Oil Mills, Schulenburg, Texas. Nitrogen content = 8.1 per cent (N × 5.4 = 43.7 per cent protein).

8. Soy Bean Flour.—From the Cereo Co., Tappan, N. Y. Nitrogen content = 8.05 per cent (N × 5.88 = 47.3 per cent protein).

9. Beef Tissue.—Prepared in this laboratory by drying the residue remaining after thoroughly extracting fresh chopped beef with water. Nitrogen content = 14.3 per cent (N × 6.25 = 89.4 per cent protein).

10. Fish Meat Meal.—A commercial product made from herring and obtained from the United States Department of Agriculture. Nitrogen content = 9.68 per cent (N × 6.25 = 60.5 per cent protein).

11. Corn Oil Cake.—A preparation of corn germs obtained from a distillery in Peoria, Ill. Nitrogen content = 2.8 per cent (N × 6.25 = 17.5 per cent protein).

12. "Vegetable Albumin Flour."—A product rich in gliadin, obtained from Arthur S. Hoyt Co., New York, and presumably consisting of the residues from the manufacture of wheat starch. Nitrogen content = 13.1 per cent (N × 5.7 = 74.7 per cent protein).

13. Brewers' Grains.—From a brewery in Milwaukee. Nitrogen content = 7.2 per cent (N × 6.25 = 45.0 per cent protein).

14. Pea Meal.—Prepared in this laboratory by grinding dry peas to a fine powder. Nitrogen content = 4.5 per cent (N × 5.88 = 26.5 per cent protein).

15. Peanut Meal.—A preparation of ground peanuts from which the oil had been expressed, obtained from the United States Department of Agriculture. Nitrogen content = 6.6 per cent (N × 5.88 = 38.8 per cent protein).

16. Distillers' Grains.—From a distillery in Peoria, Ill. Nitrogen content = 4.7 per cent (N × 6.25 = 29.4 per cent protein).

The gains in weight, which we have used as the most suitable index of growth, show wide variations for the different protein mixtures. As an illustration we may cite the following data, from substantially all of our numerous protocols, to indicate the growth of male albino rats in 42 days from an initial body weight of 60 gm. (corresponding to about 44 days of age). The food mixtures in all of the trials reported in this paper had approximately the following composition, unless otherwise indicated:

	per cent
Protein	16
"Protein-free milk"	28
Starch	28
Butter fat	18
Lard	10

Although the general trend of these results points to marked differences in the capacity of different proteins to supplement this preparation of corn gluten for the uses of growth, the figures are too variable and ill defined to permit tenable generalizations unless the conclusions are compiled from large statistical material. The wide variations with the same rations are usually due, as could easily be demonstrated from our records of the food intake, to

TABLE I.

Showing Gains of Body Weight in 42 Days From an Initial Body Weight of 60 Gm. on Otherwise Comparable Corn Gluten Food Mixtures Containing Various Protein Supplements.

Concentration of the proteins in the food.					Rat.	Gained in 42 days.
	per cent			*per cent*		*gm.*
Corn gluten	15.3				1350♂	24
"	"				1347♂	22
Corn gluten	11.4	+	lactalbumin	3.7	1445♂	106
"	"		"		1424♂	98
"	"		"		1432♂	92
"	"	12.7	"	2.5	3280♂	66
"	"	12.8	"	2.1	3276♂	48
"	"	13.0	"	1.9	3330♂	49
"	"		"		3428♂	29
"	"		"		3425♂	29
"	"		"		3340♂	26
Corn gluten	10.2	+	"milkalbumin"	5.8	2111♂	102
"	"		"		2109♂	89
"	"	11.4	"	4.4	2045♂	74
"	"		"		2032♂	60
Corn gluten	11.7	+	casein	4.1	3423♂	65
"	"		"		3433♂	62
"	"		"		3285♂	55
"	"		"		3292♂	22
"	"	13.0	"	2.7	3527♂	51
"	"		"		3575♂	36
"	"		"		3517♂	35
Corn gluten	7.8	+	edestin	8.4	3286♂	48
"	"		"		3438♂	29
"	"		"		3370♂	28
"	"		"		3424♂	18
"	"	11.7	"	4.2	3296♂	41
"	"		"		3289♂	28

TABLE I—*Continued.*

Concentration of the proteins in the food.							Rat.	Gained in 42 days.
		per cent				*per cent*		*gm.*
Corn gluten		7.8 +	cottonseed	protein		7.2	3576♂	54
"	"		"	"			3518♂	36
"	"	10.4	"	"		4.8	3394♂	44
"	"		"	"			3393♂	39
"	"	11.7	"	"		3.6	3390♂	43
"	"		"	"			3396♂	43
Corn gluten		7.8 +	cottonseed	flour		9.0	3268♂	103
"	"		"	"			3267♂	74
"	"	10.1	"	"		6.0	3290♂	46
"	"		"	"			3314♂	46
"	"	11.4	"	"		4.4	3367♂	58
"	"		"	"			3369♂	51
"	"		"	"			3429♂	44
"	"		"	"			3435♂	24
"	"	12.5	"	"		3.6	3513♂	47
"	"		"	"			3522♂	36
Corn gluten		7.6 +	soy bean	flour		9.0	3395♂	112
"	"		"	"	"		3403♂	85
"	"	10.4	"	"	"	6.0	3455♂	111
"	"		"	"	"		3457♂	94
"	"	11.4	"	"	"	4.5	3443♂	38
"	"		"	"	"		3458♂	36
Corn gluten		10.4 +	beef	tissue		5.4	3391♂	59
"	"		"	"			3399♂	52
"	"	11.7	"	"		4.0	3325♂	44
"	"		"	"			3405♂	44
"	"		"	"			3323♂	36
"	"	12.2	"	"		3.2	3309♂	66
"	"		"	"			3324♂	37

TABLE I—*Concluded.*

Concentration of the proteins in the food.							Rat.	Gained in 42 days.
per cent						*per cent*		*gm.*
Corn gluten	10.4	+	fish meat meal			6.1	3326♂	54
"	"		"	"	"		3310♂	42
Corn gluten	11.2	+	corn oil cake			4.6	3331♂	49
"	"		"	"	"		3339♂	41
Corn gluten	5.4	+	"vegetable albumin flour"			10.5	3417♂	23
"	"		"	"	"		3422♂	22
"	"	7.6	"	"	"	7.5	3263♀	26
"	"		"	"	"		3264♀	16
Corn gluten	7.6	+	brewers' grains			9.1	3332♂	36
"	"		"	"			3311♂	31
"	"	10.4	"	"		5.9	2815♀	31
"	"		"	"			2821♂	30
"	"		"	"			2809♂	25
Corn gluten	7.8	+	pea meal			7.9	3553♂	79
"	"		"	"			3486♂	53
Corn gluten	7.8	+	peanut meal			8.2	3540♂	98
"	"		"	"			3491♂	53

the unlike food consumption of individual animals. In a general way it is nevertheless apparent that among the proteins studied small addenda of lactalbumin far surpass in their supplementing efficiency decidedly larger proportions of other proteins or high protein feeds tested. Characteristic examples of supplementary inadequacy, on the other hand, are demonstrated by the small gains produced by large replacements with "vegetable albumin flour" (consisting largely of gliadin) or brewers' grains (consisting largely of residues of corn and barley proteins). Our specimens of distillers' grains (largely composed of corn protein) have shown no superiority whatever over the corn gluten itself, for the nutrition during growth.

For purposes of more strict comparison, therefore, we have selected from our numerous experiments those records in which the

food intakes during a given length of time were fairly uniform. According to our curve of normal growth a male rat weighing 60 gm. should gain 50 gm. in about 3 weeks. From the food intake data of a number of rats which have made this amount of growth in the specified time it appears that they required, on an average, 150 gm. of our foods. Taking this figure as a standard for the 3 weeks' gain indicated, and eliminating records in which the food intake showed a deviation of more than ±10 per cent, we obtain the data give in Table II.

On a *less adequate* protein mixture of the same calorific value a 60 gm. rat may require 6, 9, or even 12 weeks to gain 50 gm. in weight. From accumulated data concerning the food intake of growing rats we have estimated that the approximate amounts of food necessary to make these *slower* gains referred to are about 260, 385, and 500 gm. of food respectively. Tables III, IV, and V show the gains in weight of rats for which the food intake varied not more than ±10 per cent from these average figures. In a few protocols in these tables figures for food intake showing a deviation of somewhat more than ±10 per cent from the accepted standard have been introduced. Although they are not so strictly comparable with the rest of the records here published, they nevertheless have a comparative significance in that they represent either a gain, normal for the particular group under discussion, on a decidedly smaller food intake, or a failure to make the expected gain even when decidedly larger quantities of food were consumed.

An inspection of these tables shows that lactalbumin is by far the most efficient supplement to corn gluten of any that we have tried. When as little as one-quarter of the corn gluten is replaced by lactalbumin a rat is able to make growth at a perfectly normal rate. (Table II.) The only other supplements among those mentioned which are equally efficient in small replacements are the commercial "milkalbumin" and the soy bean flour—both effective in proportions of approximately two-thirds corn gluten to one-third of the better protein. When even about one-half of the protein is furnished as casein or edestin the otherwise similar food mixture is incapable of promoting normal growth, with food intakes of this standard magnitude.

TABLE II.

Growth of 60 Gm. Rats in 3 Weeks. The Total Food Intake in All These Trials Was Comparable and Ranged Approximately from 135 to 165 Gm. (150 ± 10 Per Cent).

Experiments with rations containing approximately 16 per cent of protein.

Protein concentrates and proteins used in the food.	Concentration of protein in food.		Rat.	Total food intake.	Body weight gained.*
	per cent			*gm.*	*gm.*
Corn gluten	11.4	15.1	1424♂	157	56
Lactalbumin	3.7				
"			1445♂	150	54
"			1432♂	159	49
Corn gluten	10.2	16.0	2111♂	158	49
"Milkalbumin"	5.8				
"			2109♂	141	45
"			2108♀	148	**40**
Corn gluten	10.4	16.4	3455♂	156	51
Soy bean flour	6.0				
"			3457♂	152	46
Corn gluten	7.6	16.6	3395♂	151	52
Soy bean flour	9.0				
Corn gluten	7.6	16.6	3268♂	149	48
Cottonseed flour	9.0				
Corn gluten	7.8	16.0	3540♂	159	49
Peanut meal	8.2				
Corn gluten	7.6	15.7	1353♀	153	**41**
Casein	8.1				
Brewers' grains		18.1	2775♂	148	**27**
"Vegetable albumin flour"		14.9	2676♂	148	**26**

* In all cases where the expected gain of 45 to 55 gm. (50 gm. ± 10 per cent) in body weight failed to be made, the figures are printed in bold-faced type.

TABLE II—*Concluded.*

Experiments with rations containing less than 15 per cent of protein.

Protein concentrates and proteins used in the food.	Concentration of protein in food.	Rat.	Total food intake.	Body weight gained.*
	per cent		*gm.*	*gm.*
Edestin	14.0	2507 ♂	139	**33**
Casein	10.8	2623 ♂	156	51
"		2630 ♂	167	58
"Milkalbumin"	9.6	2722 ♂	145	**41**
"		2716 ♂	142	**32**
Lactalbumin	9.1	2474 ♂	144	52
Corn oil cake	9.1	2929 ♂	168	**39**
Cottonseed flour	9.0	3368 ♂	140	**35**
Edestin	8.4	2609 ♂	156	**28**
Casein	8.1	2595 ♂	138	**22**
Corn gluten 5.2 Lactalbumin 2.5	7.7	2270 ♂	168	**32**
Lactalbumin	7.4	2115 ♂	135	**30**
Lactalbumin	6.6	2625 ♂	182	45
"		2631 ♂	166	**40**
Lactalbumin	5.0	2606 ♂	157	**21**

In the experiments involving less rapid gain in weight (Table III) the relative superiority of lactalbumin is again demonstrated; for no other protein among those already tested by addition to corn gluten in equally small proportion produced a gain of 50 gm. in 6 weeks.

The growth of Rat 3331 in Table III demonstrates the difference in nutritive value between the proteins of the embryo and those of the endosperm in the corn kernel; for when about one-fourth of the protein was furnished in the form of the "corn oil cake" the rat was able to make twice as much gain in 6 weeks as

TABLE III.

Growth of 60 Gm. Rats in 6 Weeks. The Total Food Intake in All These Trials Was Comparable and Ranged Approximately from 235 to 285 Gm. (260 Gm. ± 10 Per Cent).

Experiments with rations containing approximately 16 per cent of protein.

Protein concentrates and proteins used in the food.	Concentration of protein in food.	Rat.	Total food intake.	Body weight gained.*
	per cent		*gm.*	*gm.*
Corn gluten 13.0 Lactalbumin 1.9	14.9	3330♂	248	49
Corn gluten 12.8 Lactalbumin 2.1	14.9	3281♀	267	49
"		3276♂	266	48
Corn gluten 12.7 Lactalbumin 2.5	15.2	3280♂	257	66
Corn gluten 13.0 Casein 2.7	15.7	3527♂	266	51
Corn gluten 12.2 Beef tissue 3.2	15.4	3324♂	236	**37**
Corn gluten 11.4 "Milkalbumin" 4.4	15.8	2045♂	258	74
"		2032♂	272	60
"		2048♀	238	56
Corn gluten 11.7 Casein 4.1	15.8	3423♂	285	65
"		3433♂	278	62
"		3285♂	235	55
Corn gluten 11.4 Cottonseed flour 4.4	15.8	3367♂	284	58
"		3369♂	251	51
"		3429♂	263	**44**
Corn gluten 11.2 Corn oil cake 4.6	15.8	3331♂	284	49
"		3339♂	284	**41**
Corn gluten 11.4 Beef tissue 4.0	15.4	3325♂	260	**44**

TABLE III—*Continued.*

Protein concentrates and proteins used in the food.	Concentration of protein in food.	Rat.	Total food intake.	Body weight gained.
	per cent		*gm.*	*gm.*
Corn gluten 11.7 Cottonseed protein 3.6	15.3	3390♂	309	**43**
"		3394♂	330	**43**
Corn gluten 11.7 Edestin 4.2	15.9	3296♂	289	**41**
Corn gluten 10.4 Beef tissue 5.4	15.8	3391♂	270±	59
"		3399♂	245	52
Corn gluten 10.1 Cottonseed flour 6.0	16.1	3290♂	255	46
"		3314♂	267	46
Corn gluten 10.4 Brewers' grains 5.9	16.3	2815♀	245	**31**
Corn gluten 10.4 Cottonseed protein 4.8	15.2	3393♂	240±	**39**
"		3394♂	295±	**44**
Corn gluten 7.8 Cottonseed protein 7.2	15.0	3576♂	268	54
Corn gluten 7.8 Edestin 8.4	16.2	3286♂	242	48
Corn gluten 7.8 Pea meal 7.9	15.7	3486♂	279	53
Corn gluten 7.6 "Vegetable albumin flour" 7.5	15.1	3263♀	237	**26**
Brewers' grains	18.1	2791♂	277	58
"		2787♂	270	54
"Vegetable albumin flour"	14.9	2769♂	283	**40**
"		2764♂	266	**38**
"		2715♂	261	**38**

TABLE III—*Concluded.*

Experiments with rations containing less than 15 per cent of protein.

Protein concentrates and proteins used in food.	Concentration of protein in food.	Rat.	Total food intake.	Body weight gained.*
	per cent		*gm.*	*gm.*
Edestin	11.2	2176♂	247	46
"		2829♂	238	45
Casein	10.8	2117♂	249	58
"		2830♂	242	54
"		2828♂	240	52
"		2831♂	248	**43**
Lactalbumin	9.9	2786♂	226	58
"		2782♂	233	52
"		2772♂	238	49
Lactalbumin	9.1	2478♂	257	54
"		2931♂	284	63
Corn oil cake	9.1	2937♂	289	45
Cottonseed flour	9.0	3350♂	288	57
Soy bean flour	9.0	3401♂	276	48
"		3404♂	268	**42**
Edestin	8.4	2598♂	266	**27**
"		2110♂	240	**25**
Casein	8.1	2595♂	278	**40**
"		2051♂	246	**32**
Lactalbumin	8.0	3192♂	252	55
"		3194♂	238	54
Beef tissue	8.0	2914♂	282	51
"Vegetable albumin flour"	7.5	2811♂	297	**24**
Lactalbumin	7.4	2210♂	282	62
"		2207♂	276	48
Lactalbumin	5.0	2596♂	285	**44**

* In all cases where the expected gain of 45 to 55 gm. (50 gm. ± 10 per cent) in body weight failed to be made, the figures are printed in boldfaced type.

TABLE IV.

Growth of 60 Gm. Rats in 9 Weeks. The Total Food Intake in All These Trials Was Comparable and Ranged Approximately from 345 to 425 Gm. (385 Gm. ± 10 Per Cent).

Experiments with rations containing approximately 16 per cent of protein.

Protein concentrates and proteins used in the food.	Concentration of protein in food.		Rat.	Total food intake.	Body weight gained.*
	per cent			*gm.*	*gm.*
Corn gluten	13.0	14.9	3425♂	362	**39**
Lactalbumin	1.9				
Corn gluten	13.0	15.7	3517♂	387±	61
Casein	2.7				
"			3575♂	395±	50
Corn gluten	12.2	15.4	3324♂	380	55
Beef tissue	3.2				
Corn gluten	12.5	16.1	3522♂	353	48
Cottonseed flour	3.6				
Corn gluten	11.4	15.9	3443♂	369	65
Soy bean flour	4.5				
"			3458♂	348	50
Corn gluten	11.7	15.7	3405♂	374±	64
Beef tissue	4.0				
"			3323♂	350	60
"			3325♂	406	60
Corn gluten	7.6	16.7	3332♂	362±	46
Brewers' grains	9.1				
Corn gluten	7.8	15.0	3518♂	344	48
Cottonseed protein	7.2				
Corn gluten	7.8	16.2	3438♂	323	46
Edestin	8.4				
"Vegetable albumin flour"		14.9	2715♂	390	48

TABLE IV—*Concluded.*

Experiments with rations containing less than 15 per cent of protein.

Protein concentrates and proteins used in the food.	Concentration of protein in food.	Rat.	Total food intake.	Body weight gained.*
	per cent		*gm.*	*gm.*
Edestin	14.0	2511♂	400	59
Brewers' grains	13.3	2801♂	411	51
Edestin	11.2	2120♂	354	64
"		2176♂	399	72
Edestin	8.0	3212♂	385	51
"		3210♂	385	48
Casein	8.1	2051♂	364	45
"Vegetable albumin flour"	7.5	2811♂	486	**43**
"		2813♂	442	**34**
Lactalbumin	7.4	2123♂	318	70
Cottonseed flour	6.1	3420♂	422	50
"		3415♂	402	**44**
Edestin	5.6	2608♂	469	**38**
"		2616♂	427	**33**
Casein	5.4	2509♂	385	**32**
Corn gluten 3.5 Lactalbumin 1.7	5.2	2486♂	422	**42**
"		2487♂	440	**38**
Lactalbumin	3.7	2044♂	350	45
"		2049♀	385	45
Lactalbumin	2.1	2203♂	367	**18**

* In all cases where the expected gain of 45 to 55 gm. (50 gm. ± 10 per cent) in body weight failed to be made, the figures are printed in boldfaced type.

TABLE V.

Growth of 60 Gm. Rats in 12 Weeks. The Total Food Intake in All These Trials Was Comparable and Ranged from Approximately 450 to 550 Gm. (500 Gm. ± 10 Per Cent).

Experiments with rations containing approximately 16 per cent of protein.

Protein concentrates and proteins used in the food.	Concentration of protein in food.	Rat.	Total food intake.	Body weight gained.*
	per cent		*gm.*	*gm.*
Corn gluten	15.3	1350♂	492	48
"		1347♂	475	**34**
Corn gluten 13.0 Lactalbumin 1.9	14.9	3428♂	399	47
"		3340♂	433	48
Corn gluten 11.7 Casein 4.1	15.8	3292♂	468	50
Corn gluten 11.7 Edestin 4.2	15.9	3289♂	510	53
Corn gluten 11.4 Cottonseed flour 4.4	15.8	3435♂	450±	49
Corn gluten 7.6 Brewers' grains 9.1	16.7	3332♂	480±	58
Corn gluten 5.4 "Vegetable albumin flour" 10.5	15.9	3417♂	449	50

Experiments with rations containing less than 15 per cent of protein.

Edestin	8.4	2110♀	494	62
Casein	5.4	2509♂	550	50
Edestin	4.2	2114♀	485	**33**
Lactalbumin	3.7	2044♂	479	55
"		2049♀	509	50
Lactalbumin	2.1	2203♂	499	**29**
Lactalbumin	1.9	2180♂	450	**−8**

* In all cases where the expected gain of 45 to 55 gm. (50 gm. ± 10 per cent) in body weight failed to be made, the figures are printed in bold-faced type.

when all of the protein came from the endosperm. (Rats 1350 and 1347, Table I.) McCollum[8] has demonstrated a similarly high efficiency in promoting growth for the proteins of the wheat embryo.

These statistics also demonstrate the pronounced efficiency of the proteins of both the cottonseed and the soy bean as supplements to corn gluten, compared with the relative inefficiency of the proteins in brewers' grains, and "vegetable albumin flour;" for the replacement of about one-fifth or one-fourth of the corn gluten protein with the protein of the cottonseed flour or soy bean flour respectively enables a rat to make more rapid growth than substitutions of one-half or even two-thirds of the total protein by the proteins of brewers' grains and "vegetable albumin flour." (Tables IV and V.)

That the small addenda of these "better" proteins actually *supplement* the corn gluten, instead of themselves furnishing all of the protein available for growth, is shown by a comparison of the growth of rats on mixtures containing small amounts of these proteins with that of rats on equivalent or even larger quantities of the same proteins alone, *i.e.*, without corn gluten. Thus Rat 3330 (Table III) on a mixture of seven-eighths corn gluten and one-eighth lactalbumin gained 49 gm. in 6 weeks, whereas Rat 2203 (Table V) on a calorifically comparable food containing practically the same amount of lactalbumin alone—2.1 per cent—gained only 29 gm. in 12 weeks. Furthermore, Rat 3527 (Table III) on a mixture of about five-sixths corn gluten and one-sixth casein gained 51 gm. in 6 weeks, while Rat 2595 on a food containing 8.1 per cent of casein (three times as much of this protein as Rat 3527 had) gained only 40 gm. in the same time, the total food intakes of the two rats being almost identical.

A careful study of the tables presented will show numerous other instances of the efficiency of combinations of proteins in proportions in which either one alone fails to produce comparable growth. A few of these contrasts are selected for direct comparison.

Since the non-protein components of the ration used when mixed with suitable protein have been proved adequate for the

[8] McCollum, E. V., Simmonds, N., and Pitz, W., *J. Biol. Chem.*, 1916, xxv, 105.

TABLE VI.

Rat	Protein concentrates and proteins used in the food.	Concentration of protein in food.	Body weight gained.	Time.
		per cent	*gm.*	*wks.*
1424♂	Corn gluten	11.4	56	3
	Lactalbumin	3.7		
2044♂	Lactalbumin	3.7	55	12
3527♂	Corn gluten	13.0	51	6
	Casein	2.7		
2509♂	Casein	5.4	50	12
3289♂	Corn gluten	11.7	53	12
	Edestin	4.2		
2114♀	Edestin	4.2	33	12
3290♂	Corn gluten	10.1	46	6
	Cottonseed flour	6.0		
3522♂	Corn gluten	12.5	48	9
	Cottonseed flour	3.6		
3420♂	Cottonseed flour	6.0	50	9
3455♂	Corn gluten	10.4	51	3
	Soy bean flour	6.0		
3443♂	Corn gluten	12.3	38	6
	Soy bean flour	4.5		
3401♂	Soy bean flour	9.0	48	6
3331♂	Corn gluten	11.2	49	6
	Corn oil cake	4.5		
3410♂	Corn oil cake	4.5	10	6

growth of rats, any nutritive deficiency in the diets under discussion may be referred to the amino-acid make-up of the proteins employed. Previous experiments have shown that the inefficiency of zein which forms 31.3 per cent of the protein of corn gluten is due to a lack of lysine and tryptophane among its products of hydrolysis.[4] That the inefficiency of corn gluten is also due to this cause is demonstrated by recent experiments not heretofore published (Chart I). The addition of either amino-acid by itself failed to promote any noticeable increase in the rate of growth. Bearing this fact in mind, it is easy to understand why the brewers' and distillers' grains and the "vegetable albumin flour" have

proved such inadequate supplements to corn gluten. The brewers' and distillers' grains, consisting, as they do, very largely of corn proteins, are unable materially to increase the content of tryptophane and lysine in the food. The superiority of the brewers' grains as compared with the brand of distillers' grains used by us is presumably due to the presence of the barley proteins and perhaps the proteins of the grain embryo which furnish some addition of these essential amino-acids. The "vegetable albumin flour," because of its large content of gliadin which is low in lysine,[9] is unable to supplement corn gluten effectively; for no matter how rich in tryptophane the food may be, unless a certain minimum amount of lysine is present, growth at a normal rate cannot take place.[10] The "law of minimum" for amino-acids is thus seen to determine, to a large extent at least, the relative value of different proteins and protein concentrates when used to supplement corn gluten.

If the problem is considered from a purely practical standpoint it is obvious that the more rapid gains may be the cheaper in the end, even though the food used is more expensive than that which is capable of inducing only slow growth; for by rapid growth the cost of maintenance is greatly diminished. For example, Rat 1424 which required 157 gm. of food to grow 56 gm. in *3 weeks* made a much cheaper gain than Rat 3332 which required 480 gm. of food to grow 58 gm. in *12 weeks*. Moreover, a small amount of one protein may be just as efficient for growth as a larger amount of another protein or even sometimes more efficient. A few illustrative examples of this are given below.

Inasmuch as protein is ordinarily the most expensive component of a ration, the essence of economical feeding of growing animals consists in the selection of those proteins which induce the maximum amount of gain per gram of protein ingested, provided that the other necessities of the diet are furnished in suitable amounts. The findings recorded in the present series of experiments apply solely to relative values of the *protein* factors in the

[9] Gliadin contains approximately 0.92 per cent lysine; see Osborne, T. B., Van Slyke, D. D., Leavenworth, C. S., and Vinograd, M., *J. Biol. Chem.*, 1915, xxii, 259.

[10] Osborne and Mendel, *J. Biol. Chem.*, 1916, xxv, 1.

TABLE VII.

A Comparison of the Relative Efficiency of Small Amounts of Some Proteins with Larger Amounts of Other Proteins in Promoting Growth.

Rat.	Protein concentrates and proteins used in the food.	Concentration of protein in food.		Total intake.		Body weight gained.	Time.
				Food.	Protein.		
		per cent		*gm.*	*gm.*	*gm.*	*wks.*
2276♂	Corn gluten	5.2	7.7	304	23.4	68	6
	Lactalbumin	2.5					
3417♂	Corn gluten	5.4	15.9	449	70.9	50	12
	"Vegetable albumin flour"	10.5					
3350♂	Cottonseed flour		9.0	288	25.9	57	6
2791♂	Brewers' grains		18.1	277	50.1	58	6
2110♂	Edestin		8.4	494	41.5	62	12
1350♂	Corn gluten		15.3	492	75.2	48	12
3420♂	Cottonseed flour		6.1	422	25.7	50	9
2801♂	Brewers' grains		13.3	411	54.6	51	9
2486♂	Corn gluten	3.5	5.2	422	21.9	42	9
	Lactalbumin	1.7					
2811♂	"Vegetable albumin flour"		7.5	486	36.4	43	9
2596♂	Lactalbumin		5.0	285	14.2	44	6
2769♂	"Vegetable albumin flour"		14.9	283	42.2	40	6
3401♂	Soy bean flour		9.0	276	24.8	48	6
3486♂	Corn gluten	7.8	15.7	279	43.8	53	6
	Pea meal	7.9					

feeds used. They do not give evidence as to the relative value of their other dietary constituents.

Another suggestive comparison of the relative merits of a few commercial protein concentrates as supplements to corn gluten is furnished by the records of two comparable periods of observation on the same rats in each case, tabulated below.

These rats were changed to the more suitable food mixture containing corn gluten with soy bean or cottonseed flour, after a preliminary period on corn gluten + distillers' grains or "vegetable albumin flour." On the former foods the gains were distinctly greater than on the latter, with approximately the same

TABLE VIII.

Rat.	Concentration of protein in the food.				Body weight gained.	Food intake.	Time.
		per cent		*per cent*	*gm.*	*gm.*	*wks.*
3256 ♀	Corn gluten	7.6	+ distillers' grains	8.0	−1	155	4
	"	"	cottonseed flour	9.0	+34	168	4
3257 ♀	"	"	distillers' grains	8.0	10	162	4
	"	"	soy bean flour	9.0	23	138	4
3263 ♀	"	"	"vegetable albumin flour"	7.5	26	276	7
	"	"	cottonseed flour	9.0	33	268	7
3264 ♀	"	"	"vegetable albumin flour"	7.5	17	246	7
	"	"	soy bean flour	9.0	32	243	7

food intakes, although during the second period the rats had become appreciably larger than in the first period, and might reasonably be expected to require more food.

SUMMARY.

Data regarding the growth of rats are presented to show the relative nutritive values of a number of proteins and protein "concentrates" when used to supplement corn gluten in an otherwise adequate ration. The products studied included casein, lactalbumin, edestin, cottonseed protein, cottonseed flour, soy bean flour, "milkalbumin," beef tissue, fish meat meal, corn oil cake, "vegetable albumin flour," brewers' grains, distillers' grains, pea meal, and peanut meal. Such food mixtures of approximately the same nitrogen and calorific content vary greatly in their efficiency for promoting growth, in accordance with both the character and the proportion of the protein supplement employed.

The efficiency of these supplements presumably depends essentially upon their relative content of lysine and tryptophane; for the addition of these amino-acids, either as such, or in the form of proteins yielding them, renders corn gluten suitable for growth.

Of the various proteins employed to supplement the inefficient corn gluten, lactalbumin is by far the most effective. Satis-

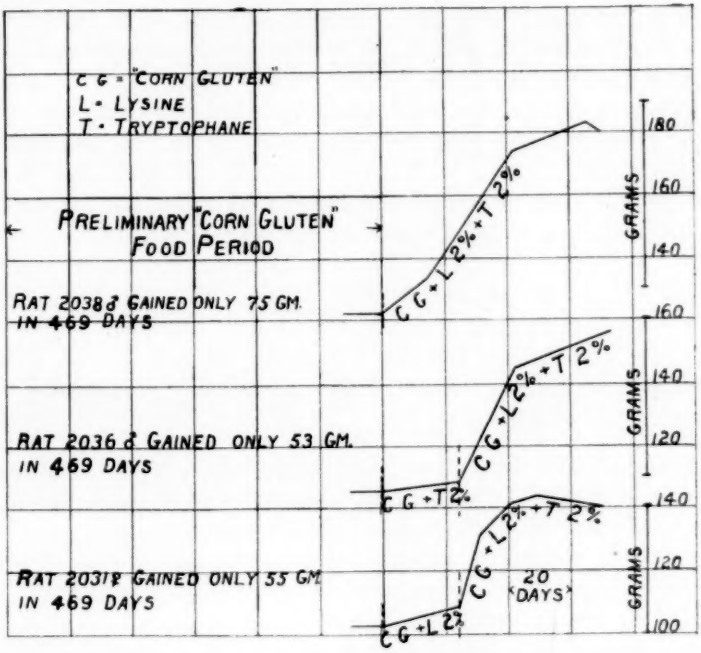
C G = "CORN GLUTEN"
L = LYSINE
T = TRYPTOPHANE
PRELIMINARY "CORN GLUTEN" FOOD PERIOD
RAT 2038 ♂ GAINED ONLY 75 GM. IN 469 DAYS
RAT 2036 ♂ GAINED ONLY 53 GM. IN 469 DAYS
RAT 2031 ♀ GAINED ONLY 55 GM. IN 469 DAYS
C G + L 2% + T 2%
C G + T 2%
C G + L 2%
20 DAYS
GRAMS
180
160
140
120
100

CHART I. Showing the resumption of growth when the amino-acids lysine and tryptophane were added to a diet containing corn gluten as the source of nitrogen. Neither of these amino-acids alone sufficed to induce an increased rate of growth when added to the corn gluten ration deficient in both of them. The foods consisted of:

	Preliminary stunting periods. *per cent*	Subsequent periods. *per cent*
Corn gluten	25–38	38.00
Amino-acids	0	0.36–1.72
"Protein-free milk"	28	28
Starch	2–15	1.64–1.28
Butter fat	3–18	18
Lard	14–29	14

factory growth is produced with smaller quantities of this protein than of any of the others recorded. The samples of brewers' grains, distillers' grains, and "vegetable albumin flour" used were the least efficient supplements tested, presumably because of their low content of lysine.

It is evident that the small additions of the more efficient proteins actually *supplement* the corn gluten instead of themselves furnishing all of the protein used for growth, because equivalent amounts of these proteins alone in a similar ration are incapable of inducing a comparable degree of growth. Small amounts of a superior protein are often just as efficient for growth as larger amounts of a less adequate protein.

The plan of feeding and comparison here described affords a practical method of studying accurately on a small scale the comparative nutrient value of the nitrogenous components of commercial feedingstuffs.

CHOLESTEROL IN HUMAN BLOOD UNDER PATHOLOGICAL CONDITIONS.

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During the past 10 years a considerable number of observations have been published concerning the cholesterol content of the blood in various pathological conditions. A hypercholesterolemia has been noted in nephritis, diabetes, lues, pregnancy, cholelithiasis, obstructive jaundice, obesity, alcoholism, starvation, typhoid fever, many skin diseases, in malignant disease, in cachexias of various origins, and after narcosis. Cholesterol has been found in subnormal quantities in the blood of persons suffering from anemia, diseases of the liver (without jaundice), in certain cases of nephritis, in many acute infections, and in a variety of conditions "where the vitality is low."[1]

The number and variety of conditions in which an abnormal cholesterol content of the blood is reported are unconvincing, the more so as on examination of the literature it is apparent that the analytical methods employed by some of the frequently quoted clinical investigators in this field leave much to be desired. In view of the rapidly increasing importance of blood analysis, both to the biological chemist and to the clinician, it has seemed worth while, in spite of the relatively large number of papers to be found in the literature dealing with blood cholesterol in pathological conditions, to publish the following series of observations in which determinations of cholesterol have been made on the blood of persons suffering from the more common chronic and acute diseases.

The results reported have in every case been obtained by Bloor's modification[2] of the Autenrieth-Funk colorimetric method

[1] Bloor, W. R., *J. Biol. Chem.*, 1916, xxv, 577.

[2] Bloor, *J. Biol. Chem.*, 1916, xxiv, 227.

a procedure which has been selected because of the small quantity of blood required, the relative ease of manipulation, and the freedom from procedures which might lead to decomposition of cholesterol.

Bloor[1] has published a series of cholesterol figures for the blood of normal men and women as determined by the above method. In a series of nine normal women he observed a maximum blood cholesterol of 0.240 per cent and a minimum of 0.210 per cent. In a series of fourteen normal men the maximum is 0.250 per cent, the minimum 0.190 per cent. These figures are considerably higher than those ordinarily given in the literature, a fact probably due to the more complete extraction secured by the method of analysis employed.

In view of the relatively small number of normal bloods examined by Bloor it has seemed worth while to obtain a few more figures on normal persons. The bloods used in this series were obtained from the nurses and physicians in the hospital, all relatively young people, and from a few middle aged and old men and women who were convalescents, on the surgical services, from fractures and amputations.

TABLE I.

Blood Cholesterol in Normal Persons.

No.	Sex.	Cholesterol per 100 cc. of blood.	No.	Sex.	Cholesterol per 100 cc. of blood.
		mg.			*mg.*
C235	♀	167	C255	♂	255
C236	♀	179	B140	♂	250
B3	♀	200	B143	♂	198
B4	♀	194	B19	♂	240
B5	♀	225	42	♂	192
B6	♀	190	45	♂	250
C196	♂	223	46	♂	228
C230	♂	211	59	♂	208
C229	♂	210	133	♂	223
C251	♂	200	136	♂	249

From this series it is apparent that cholesterol values on normal blood may vary from 167 to 255 mg. per 100 cc. of blood, a variation somewhat wider than that given by Bloor.

In connection with the statements to be found in the literature regarding the hypercholesterolemia found in the obese, it is interesting to note that the lowest figure in Table I (167 mg.) was obtained on the blood of a woman weighing 200 pounds, while the next lowest figure (179 mg.) was obtained on the blood of a woman weighing 260 pounds.

Before beginning the collections of blood it seemed desirable to determine whether the ingestion of food, particularly food rich in lipoids, has any immediate effect on the cholesterol content of the blood. It has long been known that prolonged cholesterol feeding produces in animals an increase in the cholesterol content of the blood.[3] No statements are, however, to be found in the literature concerning the effect, if any, produced by a single lipoid-rich meal.

In order to determine this point three men were selected (convalescent fracture cases) who had sufficiently large appetites to eat a breakfast consisting of four eggs, 200 gm. of mutton chop, 50 gm. of bacon, ½ pint of cream, bread, butter, and coffee. Blood was taken just before breakfast, at which time the patients had been fasting for 15 hours, and again 3 hours after the meal.

Cholesterol per 100 cc. of blood.		mg.
L.	Fasting	250
	3 hrs. after breakfast	250
H.	Fasting	185
	3 hrs. after breakfast	181
B.	Fasting	210
	3 hrs. after breakfast	212

As will be seen from the above results the ingestion of lipoid-rich food has no immediate effect on the cholesterol content of the blood, and I have therefore felt justified in taking my samples of blood at any convenient hour, although they were in a large majority of cases taken about 4 hours after the midday meal.

Blood Cholesterol in Nephritis.

On account of the fundamental part in the formation of arteriosclerotic lesions which has been ascribed to cholesterol by some

[3] Grigaut, A., and L'Huillier, A., *Compt. rend. Soc. biol.*, 1912, lxxiii, 304. Gardner, J. A., and Lander, P. E., *Biochem. J.*, 1913, vii, 576. Luden, G., *J. Biol. Chem.*, 1916, xxvii, 273.

TABLE II.

Blood Cholesterol in Nephritis.

No.	Sex.	Age.	Diagnosis.	Per 100 cc. of blood.	
				Non-protein nitrogen.	Cholesterol.
		yrs.		*mg.*	*mg.*
51	♂	42	Chronic nephritis. Hypertension.	32	250
48	♂	35	" " Uremia. Died 10 days later.	69	257
50	♂	42	Chronic nephritis.	43	257
49	♂	38	Nephritis. Acute exacerbation following infection of hand. Died 18 hrs. after blood was taken.	187	250
205	♂		Nephritis. Hypertension. Arteriosclerosis. Much edema.	36	238
C267	♂		Nephritis. Hypertension. Arteriosclerosis.	88	213
C245	♂	53	Chronic nephritis. Hypertension. Arteriosclerosis. Retinitis. Phthalein test 20 per cent in 2 hrs.	51	292
C199	♀	45	Chronic nephritis. Arterial walls much thickened and very tortuous. Retinitis. Hypertension. Phthalein test 15 per cent in 2 hrs.	55	265
53	♂		Chronic nephritis. Hypertension.	31	245
44	♂	23	Acute "	100	232
47	♂	44	Chronic "	42	228
62	♀	60	" " Marked arteriosclerosis.	54	217
136	♀	54	Chronic nephritis.	33	238
153	♂	48	Chronic interstitial nephritis. Hypertension. Uremia. Phthalein test 1 per cent in 2 hrs. Died 2 days after blood was taken.	238	208
220	♂		Chronic nephritis. Marked arteriosclerosis. Hypertension.	48	211
129	♂		Chronic nephritis.	76	206
C231	♂	40	" " Hypertension. Albuminuric retinitis. Phthalein test 2 per cent in 2 hrs. Died 2 days later.	234	201
C173	♂	80	Chronic nephritis. Marked arteriosclerosis. Phthalein test 2 per cent in 2 hrs. Died 4 days after blood was taken.	54	211
C200	♂	19	Acute nephritis. Slight edema. Phthalein test 30 per cent in 2 hrs.	45	245

TABLE II—*Continued.*

No.	Sex.	Age.	Diagnosis.	Per 100 cc. of blood.	
				Non-protein nitrogen.	Cholesterol.
		yrs.		*mg.*	*mg.*
226	♂	52	Chronic nephritis. Marked arteriosclerosis. Slight edema.	81	208
C254	♂	40	Chronic nephritis. Phthalein test 45 per cent in 2 hrs.	71	216
54	♂		Chronic nephritis. Slight arteriosclerosis. Phthalein test 53 per cent in 2 hrs.	37	212
126	♀	16	Acute nephritis. Phthalein test 14 per cent in 2 hrs. Much edema.	75	208
225	♂	54	Chronic nephritis. Moderate edema. Phthalein test 25 per cent in 2 hrs.	71	199
118	♀	23	Chronic nephritis. Much edema.	39	166
112	♂	39	" " Very slight edema. Moderate arteriosclerotic changes. Phthalein test 5 per cent in 2 hrs.	125	178
113	♂	45	Chronic nephritis. Phthalein test 40 per cent in 2 hrs.	55	178
139	♂	65	Chronic nephritis. Moderate edema.	45	181
130	♂	53	" " Chronic plumbism. Marked arteriosclerotic changes.	65	169
206	♀	33	Chronic nephritis. Much edema.	36	165
56	♀	62	" " Moderate arteriosclerotic changes.	62	146
129		52	Chronic nephritis. Moderate edema.	76	206
140			Same patient as above after 3 weeks' treatment.	40	158
44		23	Acute nephritis. Moderate edema. Phthalein test 15 per cent in 2 hrs.	100	232
B82			Same patient 28 days later.	50	192
B99			" " 42 " "	41	245
C304			" " 63 " " Phthalein test 50 per cent in 2 hrs.	38	225
C221		40	Chronic nephritis. Hypertension. Albuminuric retinitis. Phthalein test 2 per cent in 2 hrs.	111	181
C231			Same patient as above 6 days later. Blood taken while in uremic convulsion. Died in 36 hrs.	234	201

TABLE II—*Concluded.*

No.	Sex.	Age.	Diagnosis.	Per 100 cc. of blood.	
				Non-protein nitrogen.	Cholesterol.
		yrs.		*mg.*	*mg.*
C246		38	Chronic nephritis. Arteriosclerosis. Uremia. Phthalein test 1 per cent in 2 hrs.	89	231
C260			Same patient as above 4 days later; growing steadily worse.	192	190
C265			Same patient 6 days later. Died 24 hrs. after this blood sample was taken.	256	194
C307			Same patient, blood taken 1 hr. before death.	298	187
C253		20	Acute nephritis. No hypertension. No arteriosclerotic changes. Albuminuric retinitis. Phthalein test 10 per cent in 2 hrs. Very severe edema.	42	475
C302			Same patient 4 weeks later. No improvement.	40	606

TABLE III.

Blood Cholesterol in Cardiorenal Disease.

No.	Age.	Diagnosis.	Per 100 cc. of blood.	
			Non-protein nitrogen.	Cholesterol.
	yrs.		*mg.*	*mg.*
43	65	Chronic nephritis. Mitral regurgitation.	50	250
55	48	" " " "		181
57	59	Cardiorenal disease. Marked arteriosclerosis.		178
58	56	Chronic nephritis. Mitral stenosis and regurgitation.		203
60	66	Chronic nephritis. Chronic endocarditis.		166
61	55	Cardiorenal disease.		166
134	48	" "	44	160
137	67	" " Marked edema. Arterial walls palpable.	48	178
138	51	Cardiorenal disease. Marked edema. Arterial walls show slight sclerosis. Phthalein test 16 per cent in 2 hrs.	39	227
141	68	Cardiorenal disease.	41	200
152	70	Chronic nephritis. Mitral disease.	55	238
C222	58	Chronic nephritis. Mitral regurgitation. Arteriosclerosis. Cardiac hypertrophy. Phthalein test 15 per cent in 2 hrs.	65	174

TABLE IV.

Blood Cholesterol in Cardiac Disease.

No.	Age.	Diagnosis.	Per 100 cc. of blood	
			Non-protein nitrogen.	Cholesterol.
	yrs.		*mg.*	*mg.*
155		Mitral regurgitation. Decompensation.	38	279
95		Aortic disease. Marked decompensation.	37	300
97		Mitral stenosis. Decompensation.	37	193
98		Mitral regurgitation. Decompensation. Ascites.	37	217
99		Chronic endocarditis. Decompensation.	45	250
100		" "		
101		" "		176
102		Aortic disease. Decompensation.		225
72		Mitral stenosis and regurgitation.	30	186
218		" " " "		176
C233	65	Aortic and mitral disease. Arteriosclerosis. Angina pectoris.	40	268
C252	58	Valvular disease. Decompensation.		240
C195	50	Mitral regurgitation. Decompensation.	43	222
C171	28	Aortic and mitral disease.		141
C268	50	Mitral regurgitation. Decompensation.	38	250

writers, much attention has been given to the collection of quantitative data concerning the occurrence of this body in nephritic blood.

In general, the opinion has been that a hypercholesterolemia occurs with great frequency in patients with chronic Bright's disease, cardiorenal disease, arteriosclerosis, and related conditions.[4] According to these investigators, however, this hypercholesterolemia is not a retention phenomenon, as shown by the fact that in patients showing a high content of urea in the blood, the cholesterol figures tend to fall, and may even become subnormal (Schmidt), while in three cases it was noted that a rise in the non-protein nitrogen of the blood was coincident with a fall in the cholesterol content (Widal[4]).

[4] Chauffard, A., Laroche, G., and Grigaut, A., *Compt. rend. Soc. biol.*, 1911, lxx, 108. Widal, F., Weill, A., and Laudat. M., *Semaine méd.*, 1912, xxxii, 529. Schmidt, H. B., *Arch. Int. Med.*, 1914, xiii, 127. Henes, E., *Deutsch. Arch. klin. Med.*, 1913, cxi, 122.

The above results do not confirm the findings of the earlier investigators concerning the high cholesterol content of nephritic blood. In one case only was a hypercholesterolemia observed. It should be pointed out, however, that these patients were all on a "nephritic diet" containing no meat, and few eggs or other foodstuffs of a high lipoid content. In the four cases in which a series of determinations were made at intervals during the progress of the disease there is no parallelism to be noted between the non-protein nitrogen and the cholesterol content of the blood.

Blood Cholesterol in Syphilis.

It has been reported that blood cholesterol is present in increased quantities in the blood of syphilitics[5] and the suggestion has even been made that this increased lipoid content may account in part at least for the "Wassermann reaction" given by luetic sera. On the other hand, Stein[6] has reported the cholesterol content of syphilitic blood to be normal in primary cases and low in secondary infections.

TABLE V.

Blood Cholesterol in Syphilis.

No.	Sex.	Age.	Diagnosis.	Cholesterol per 100 cc. of blood.
		yrs.		*mg.*
150	♂		Gastric syphilis.	250
14	♂		Enlarged liver. Wassermann strongly positive.	175
C42	♂		Syphilis.	173
C43	♂	33	Early tertiary lesion.	135
C248	♀	22	" primary "	166
C250	♀	15	Congenital syphilis.	170
C252	♀	16	" "	250
C220	♂	39	Late secondary lesion.	150
C237	♂	56	Syphilis. Peptic ulcer.	151
C239	♂	38	Tabes. Gastric ulcer.	238
194	♀		Syphilitic ulcer of the leg.	167
201	♀		" "	238
C264	♂		Gastric syphilis.	226
C237	♂		Syphilis of central nervous system.	158

[5] Klein, W., and Dinkin, L., *Z. physiol. Chem.*, 1914, xcii, 302.

[6] Stein, G., *Z. ges. exp. Med.*, 1914, iii, 309.

In the fourteen specimens of luetic blood examined no increase was observed in any case, in fact many gave extremely low cholesterol values.

Blood Cholesterol in Pregnancy.

An increased cholesterol content of the blood has been described in pregnant women.[7] In the case of twelve women in early pregnancy whose blood was examined no increase in cholesterol was noted.

TABLE VI.

Blood Cholesterol in Pregnancy.

No.	Diagnosis.	Cholesterol per 100 cc. of blood.
		mg.
C202	Normal pregnancy.	183
C203	" "	236
C204	" "	260
C205	" "	223
C206	" "	223
207	" "	221
224	" "	283
225	" "	224
201	Probable toxemia.	256
226	Normal pregnancy.	272
241	Persistent vomiting.	208
262	" "	224

The next series of observations would seem to indicate that in diabetes a hypercholesterolemia is of rare occurrence. Taken as a whole there seems to be no definite relation as far as can be judged by the analytical data presented either between the degree of acidosis and the blood cholesterol or between the blood sugar and cholesterol.[8]

[7] Herrmann, E., and Neumann, J., *Biochem. Z.*, 1912, xliii, 47. Neumann and Herrmann, *Wien. klin. Woch.*, 1911, xxiv, 411. Herrmann and Neumann, *ibid.*, 1912, xxv, 1557. Albrecht, H., and Weltmann, O., *ibid.*, 1911, xxiv, 483. Landau, M., *Deutsch. med. Woch.*, 1913, xxxix, 546.

[8] All samples of diabetic blood were taken before breakfast in order to avoid the complication of an alimentary glycosuria. The blood sugar determinations were made by Myers' modification of the Lewis-Benedict method.

TABLE VII.

Blood Cholesterol in Diabetes.

No.	Sex.	Age.	Urinary sugar.	Diacetic acid.	Blood sugar.	Cholesterol per 100 cc. of blood.
		yrs.	*per cent*		*per cent*	*mg.*
87	♂		0.8	+	0.15	186
88	♀		2.2	+	0.16	277
89	♀		Trace.	+	0.14	235
90	♂		6.2	+	0.16	292
91	♂		5.4	++	0.28	312
92	♂		1.4	+	0.13	225
93	♂		0	0	0.10	185
94	♀		Trace.	+	0.150	207
214	♂		2.0	++	0.25	332
216	♀		2.2	+	0.18	250
125	♀		0	0	0.097	172
127	♀		0	0	0.093	219
132	♂		0	0	0.125	166
120	♀		0	0	0.111	150
C216b	♀		2.0	+	0.18	274
C217	♀		0.5	+	0.164	256
C49	♂	50	0.79	+	0.19	166
C175	♀	44	1.0	++	0.19	250
C234	♂	35	2.8	++	0.28	300
C298	♀	42	2.2	+	0.31	183
C297	♀	52	0	0	0.14	314
C288	♂	44	0	0	0.17	245
C287	♀	28	2	0	0.17	189
C286	♂	33	0	0	0.16	367
C299	♂		1.4	+	0.16	205

Blood Cholesterol in Acute Infections.

But little work has been published regarding the effect of acute infections on the cholesterol content of the blood. Chauffard, Richet, and Grigaut[9] have found that in typhoid fever high cholesterol values are obtained in the late stages of the disease, whereas in pulmonary tuberculosis a subnormal amount is present.

The results presented on cases of acute infections show in the severe cases a marked diminution of the cholesterol content of the blood, which rises to normal values during convalescence. In a few very mild cases of typhoid no hypercholesterolemia was noted.

[9] Chauffard, Laroche, and Grigaut, *Compt. rend. Soc. biol.*, 1911, lxx, 70; Chauffard, A., Richet, C., and Grigaut, A., *ibid.*, 1911, lxx, 276.

TABLE VIII.

Blood Cholesterol in Acute Infections.

No.	Sex.	Diagnosis.	Cholesterol per 100 cc. of blood.
			mg.
140	♀	Typhoid, 2nd week. Severe case.	173
b2		Same patient 7 weeks later, convalescent.	205
147	♂	Typhoid, 2nd week. Mild case.	228
		Same patient. Late convalescence, 6 weeks later.	226
	♂	Typhoid, 3rd week. Mild case.	205
		Same patient 5 weeks later, convalescent.	222
	♂	Typhoid 3rd week. Severe case. Relapse.	145
		Same patient 9 weeks later, convalescent.	168
C151	♂	Typhoid, 2nd day of normal temperature. Severe case.	150
C182		Same patient 18 days later.	303
C164	♂	Typhoid, 4th day of normal temperature. Severe case.	164
		Same patient 4 weeks later.	210
146	♂	Typhoid, 4th week. Mild case.	168
149	♂	" early convalescence.	205
C284	♂	" 2nd week.	139
C281	♂	" 2nd "	139
C192	♂	Pneumonia. Pulmonary tuberculosis.	139
C300	♂	Same case convalescent, 7 weeks later.	162
C295	♂	Bronchial pneumonia.	151
C294	♂	Lobar pneumonia. Before crisis.	141
C289	♂	" " 12 hrs. after crisis.	166
C285	♂	" " Before crisis.	129
C278	♀	Bronchial pneumonia.	171
66	♂	Acute pleurisy.	279
C283	♂	" " with effusion.	145
75	♂	Pneumonia. Before crisis.	151
C214	♀	" " "	143
C215	♂	" " "	133
C216	♂	" Crisis 6 days later.	166
70	♂	Acute rheumatic fever.	169
C263	♂	" " "	150
104	♂	Septic foot and leg. Severe case.	203

Blood Cholesterol in Diseases of the Liver (Including Cholelithiasis).

Blood cholesterol determinations in cases of gall-stone disease have always been popular and numerous attempts have been made to connect the occurrence of cholelithiasis with a hypercholesterolemia.[10] In obstruction of the common duct an increase in cholesterol has been noted,[11] while in cirrhosis of the liver subnormal quantities of cholesterol have been reported.

TABLE IX.

Blood Cholesterol in Diseases of the Liver.

No.	Sex.	Diagnosis.	Cholesterol per 100 cc. of blood.
			mg.
142	♂	Cirrhosis of liver. Ascites.	166
13	♂	" " " Alcoholic. Marked jaundice.	170
272	♀	" " " Syphilitic. " "	170
C271	♂	" " " Alcoholic. " "	160
C280	♂	" " " Slight jaundice.	205
10	♂	Gall-stones (operation).	222
11	♂	" "	227
12	♀	" "	204
	♂	" " Slight jaundice.	174
C275	♀	" "	188
C274	♂	" "	150
D66	♀	" " Marked jaundice.	200
B43	♀	" " Slight "	183
B37	♀	" " " "	192
C191	♀	Abscess of liver. Marked jaundice.	154
208	♂	Catarrhal jaundice. Marked. Much bile in urine.	146
C279	♂	Chronic disease of gall-bladder. Much bile in urine.	273

The series of liver cases is not large, but it gives no indication that hypercholesterolemia is necessarily an accessory to gall-stone disease. In the nine cases of cholelithiasis observed, the presence of stones was in every case proved by operation. In the

[10] Henes, *J. Am. Med. Assn.*, 1914, lxiii, 146. Rothschild, M. A., and Rosenthal, N., *Am. J. Med. Sc.*, 1916, clii, 394.

[11] Chauffard, Laroche, and Grigaut, *Compt. rend. Soc. biol.*, 1911, lxx, 70. Schmidt, *Arch. Int. Med.*, 1914, xiii, 125.

cases examined which showed icteric symptoms of various degrees of severity no increase of blood cholesterol was noted.

Blood Cholesterol in Malignant Disease.

A hypercholesterolemia has been described in the early stages of malignant disease[12] while in advanced tumor cases where cachexia is present the cholesterol of the blood is low. The samples of blood examined taken from persons suffering from malignant disease have, however, given in every case results within, or in a few cases below, normal limits.

TABLE X.

Blood Cholesterol in Malignant Disease.

No.	Sex.	Diagnosis.	Cholesterol per 100 cc. of blood.
			mg.
16	♂	Carcinoma of transverse colon.	250
17	♂	" " stomach.	233
18	♂	Hypernephroma of tibia and humerus.	178
19	♂	Tumor of cord.	250
20	♀	Abdominal tumor.	182
21	♀	Inoperable carcinoma of breast.	195
22	♀	Malignant disease of the liver (marked jaundice).	277
23	♀	Carcinoma of stomach.	178
24	♂	Abdominal tumor.	154
143	♂	Malignant disease with multiple metastasis.	146
40	♂	Cancer of stomach (marked anemia).	130
C261	♀	Recurrent carcinoma of breast.	201
C303	♂	Abdominal tumor.	257
C290	♂	Cancer of stomach.	238

Blood Cholesterol in Diseases of the Skin.

In a study of blood cholesterol in diseases of the skin Fischl[13] has reported that in the ordinary dermatosis not accompanied by fever, such as urticaria, eczema, mycosis fungoides, etc., the cholesterol content of the blood is high, while in conditions accompanied by fever, such as erysipelas, herpes zoster, etc., a hypercholesterolemia is found.

[12] Luden, *J. Lab. and Clin. Med.*, 1916, i, 662.

[13] Fischl, F., *Wien. klin. Woch.*, 1914, xxvii, 982.

TABLE XI.

Blood Cholesterol in Diseases of the Skin.

No.	Diagnosis.	Sex.	Cholesterol per 100 cc. of blood.	No.	Diagnosis.	Sex.	Cholesterol per 100 cc. of blood.
			mg.				*mg.*
156	Eczema.	♂	173	168	Psoriasis.	♀	168
157	"	♂	196	169	"	♀	165
158	"	♀	145	177	"	♀	166
159	"	♂	147	179	"	♀	230
160	"	♂	227	180	"	♀	163
171	"	♂	168	187	"	♀	250
172	"	♂	195	231	"	♀	194
175	"	♂	168	161	Prurigo.	♂	151
182	"	♂	157	162	Erythema multiforme.	♂	231
190	" Jaundice.	♂	172	163	Mycosis fungoides.	♂	164
191	"	♀	194	164	Mycosis fungoides.	♂	146
195	"	♀	175	167	Vitiligo.	♀	216
227	"	♀	200	165	Lupus erythematosus.	♀	206
229	"	♀	194	170	Tubercular lesion.	♂	233
230	"	♂	192	204	Lupus erythematosus.	♂	200
174	" seborrheic.	♀	156	183	Lichen planus.	♀	195
166	" psoriasiform.	♀	184	189	" "	♀	234
228	Acne vulgaris.	♂	195	184	Scabies.	♂	186
185	" "	♂	208	186	Sporotrichosis.	♂	208
193	" "	♀	208	188	Actinodermatitis.	♂	238
197	" "	♂	185	192	Scleroderma.	♀	208
198	" "	♂	162	200	Herpes zoster.	♂	255
172	" "	♀	208	203	Pityriasis rosea.	♀	226
178	Furunculus.	♂	201				
199	"	♂	177				
176	Urticaria.	♂	208				
181	"	♀	208				
196	"	♀	166				
202	" (chronic).	♀	189				

The results presented in Table XI indicate that, in the main, persons suffering from the ordinary types of dermatoses show essentially normal blood cholesterol values. Of seventeen cases of eczema examined, it is to be noted that three show abnormally low values. Out of a total of seven cases of psoriasis four gave cholesterol figures approaching the minimal normal values, while the same may be said of one case of prurigo, two of mycosis fun-

goides, one of acne vulgaris, and one of urticaria. The cases of eczema and of psoriasis were on restricted diets, which may in part at least account for the low values obtained. The other cases giving subnormal figures were on an unrestricted food intake but were without exception old persons who had suffered from these diseases for many years and who were physically much below par.

Blood Cholesterol in Anemia.

Many observations have been published showing that the cholesterol content of the blood in anemic conditions is invariably low. In fact this finding has been so universal that cholesterol injections have even been tried in Italy as a cure for pernicious anemia.

The observations recorded below confirm these results. It should be noted, however, that the cholesterol figures vary relatively but not absolutely with the red cell count. That the anemia as such has no relation to the hypercholesterolemia, is suggested by Case 41, a vigorous young man who had suffered a severe hemorrhage, and who showed an absolutely normal cholesterol value with a blood count of less than 2,000,000.

Three cases are included in which marked improvement in the blood picture had taken place after treatment (transfusion). In one of these although the red cell count is more than doubled the cholesterol content of the blood decreased slightly, while in two other cases with an increase of 100 per cent in the cell content of the blood the cholesterol increased in one case only a trifle more than 50 per cent and in the other less than 20 per cent.

In connection with the cases of anemia two cases of polycythemia examined are worthy of mention on account of the normal cholesterol values present.

No. 124. Pulmonary stenosis with open red cells, 9,400,000 hemoglobin 148 per cent, cholesterol 170 mg. per 100 cc. of blood.

No. 25. Enlarged liver and spleen (cause?) red cells 9,200,000, hemoglobin 130 per cent, cholesterol 180 mg. per 100 cc. of blood.

TABLE XII.

Blood Cholesterol in Anemia.

No.	Diagnosis.	Red cells per c. mm.	Hemoglobin (Sahli).	Cholesterol per 100 cc. of blood.
			per cent	*mg.*
26	Pernicious anemia.	500,000	12	135
116	" "	1,000,000	20	126
117	" "	1,400,000	30	128
111	" "	2,200,000	55	139
114	" "	1,800,000	50	154
119	" "	900,000	30	166
118	" "	1,800,000	50	172
29	" "	2,300,000	60	169
32	" "	1,700,000	30	219
36	" "	1,200,000	35	130
36a	Same patient after treatment.	2,500,000	80	210
37	Pernicious anemia.	824,000	38	150
37a	Same patient after treatment.	2,100,000	60	130
38	Pernicious anemia.	2,000,000	30	180
38a	Same patient after treatment.	3,900,000	75	210
28	Splenic anemia.	2,200,000	30	190
C273	Banti's disease.	2,700,000	36	144
39	Leukemia.			176
C190	Lymphatic leukemia (acute).	1,300,000	26	144
143	Malignant disease with multiple metastases. Secondary anemia.	2,100,000	28	146
40	Cancer of stomach. Secondary anemia.	3,000,000	50	130
33	Tapeworm. Secondary anemia.		60	147
41	Gastric ulcer accompanied by severe hemorrhage. Secondary anemia.	1,840,000	29	208

Blood Cholesterol in Diseases of the Ductless Glands.

On account of the part ascribed to the ductless glands in the regulation of lipoid metabolism I have made a few examinations of blood from persons suffering from thyroid disease and in one case of acromegaly. As will be seen from the following result, no striking deviations from the normal values were found, al-

though it is of interest to note that four of the five cases of severe Graves' disease gave low figures, while the one case of myxedema examined showed a figure a trifle above the normal. No relation is apparent between blood sugar and blood cholesterol.

TABLE XIII.

Blood Cholesterol in Diseases of the Ductless Glands.

No.	Sex.	Diagnosis.	Blood sugar.	Cholesterol per 100 cc. of blood.
			per cent	*mg.*
76	♀	Hyperthyroid, severe case.	0.10	176
212	♂	" " "	0.096	163
C291	♂	" " "	0.10	178
C276	♀	" " "	0.11	180
C270	♀	" " "	0.11	231
C168	♀	" mild case.	0.096	238
C189	♀	" " "	0.12	247
C228	♂	" " "	0.11	186
C306	♀	" " "	0.10	257
C305	♀	" " "		225
C227	♀	Myxedema.	0.12	295
B102	♀	Acromegaly.	0.11	280

SUMMARY.

Determinations of cholesterol have been made on the blood of twenty normal individuals and of two hundred and fifty-four persons suffering from a variety of the more common diseases and including twelve in pregnancy. As a result of this work the following conclusions are drawn.

1. In nephritis, cardiorenal disease, arteriosclerosis, and cardiac disease the blood cholesterol remains at normal levels both in the early stages of the disease and when the patient is practically moribund. There is no relation between the non-protein nitrogen of the blood and the cholesterol.

2. In syphilis the blood cholesterol is not increased. In some cases it is low.

3. In twelve cases of early pregnancy no increase in blood cholesterol was noted.

4. In twenty-five diabetics, five showed a slight increase in the cholesterol content of the blood. This hypercholesterolemia bore

no constant relation to the blood sugar or to the acetone bodies or sugar of the urine.

5. In the case of the acute infections, typhoid fever, pneumonia, pleurisy, and rheumatic fever were studied. In these conditions low cholesterol values were found when the patient was very ill; in convalescence normal values were reestablished.

6. In nine cases of gall-stone disease no marked increase of blood cholesterol was found. In icterus even when severe there was no increase. In cirrhosis of the liver the values were within the lower normal limits.

7. In fourteen early cases of malignant disease the cholesterol figures were normal except in one case associated with anemia in which the value was low.

8. In diseases of the skin the blood cholesterol figures obtained were within normal limits.

9. In severe primary and secondary anemias subnormal values were obtained. No definite relation was found to exist between the number of corpuscles or hemoglobin percentage and cholesterol values.

10. In severe hyperthyroidism figures were obtained within or just below the lower normal limit. In one case of myxedema a cholesterol figure slightly above the highest normal value was found.

CONCLUSIONS.

Among the pathological conditions examined hypercholesterolemia was found to exist only in diabetes and only in a relatively small number of cases of this disease. Low cholesterol values are not characteristic of any special pathological condition but are invariably found in conditions where marked prostration or cachexia exist. Cholesterol determinations in the blood are therefore at present of no value in the clinical diagnosis or prognosis of disease.

I am indebted to my assistant, Miss Anna S. Minot, for many of the determinations recorded in this paper.

ADENINE-URACIL DINUCLEOTIDE AND THE STRUCTURE OF YEAST NUCLEIC ACID.

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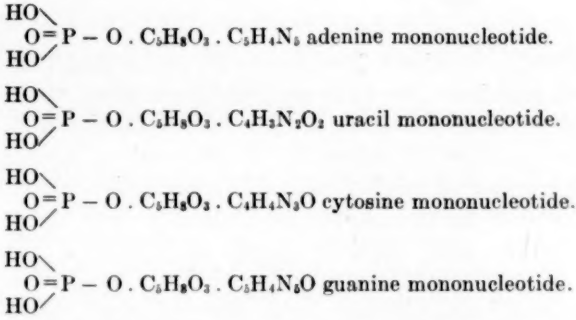
(Received for publication, December 27, 1916.)

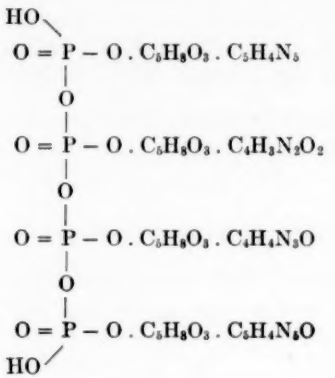
Recent investigations have necessitated the assumption that yeast nucleic acid contains groups of the four following mononucleotides.[1–7]



The gross structure of nucleic acid would therefore be determined if we could discover the points at which the four nucleotide groups are joined to one another. Only two modes of linkage have sufficient probability to require discussion. One is through the phosphoric acid groups, and the other is through the carbohydrate groups. In the one case, nucleic acid would be a substituted polyphosphoric acid, and in the other case, a substituted

[1] Levene, P. A., and Jacobs, W. A., *Ber. chem. Ges.*, 1909, xlii, 2474.
[2] Levene and Jacobs, *Ber. chem. Ges.*, 1909, xlii, 2703.
[3] Levene and Jacobs, *Ber. chem. Ges.*, 1910, xliii, 3150.
[4] Levene and Jacobs, *Ber. chem. Ges.*, 1911, xliv, 1027.
[5] Levene, P. A., and La Forge, F. B., *Ber. chem. Ges.*, 1912, xlv, 608.
[6] Jones, W., and Richards, A. E., *J. Biol. Chem.*, 1914, xvii, 71.
[7] Jones and Richards, *J. Biol. Chem.*, 1915, xx, 25.

polysaccharide. Prevailing opinion favors the former view, and nucleic acid has been assigned the formula:[3]

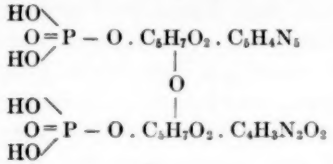


The following considerations, however, prove that this is not the correct solution. We have prepared from yeast nucleic acid a substance which by acid hydrolysis yields adenine and uracil but neither guanine nor cytosine. By ammoniacal hydrolysis it yields adenosine and uridine but neither guanosine nor cytidine. The partition of its phosphorus shows that it contains an equal number of purine and pyrimidine groups (one of each).[8] The substance is evidently adenine-uracil dinucleotide. Its physical properties are strikingly different from those of yeast nucleic acid and the yield is such as to suggest that its formation from yeast nucleic acid is quantitative.

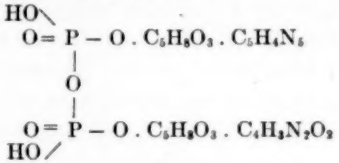
When a hot aqueous solution of the dinucleotide is treated with a solution of brucine in hot alcohol, a tetrabrucine salt is formed which is deposited in beautifully crystalline form as the solution cools. Neither the melting point nor the elementary composition of the brucine salt is changed by repeated crystallization of the substance from hot water. By acid hydrolysis it yields adenine but not guanine. Half of its phosphoric acid is easily split, half is firmly bound. Analyses for carbon, hydrogen, nitrogen, phosphorus, and brucine give sharply the values required for the formula $C_{19}H_{25}N_7P_2O_{15}.(C_{23}H_{26}N_2O_4)_4$.

[8] Jones, W., *J. Biol. Chem.*, 1916, xxiv, p. iii.

Two structural formulas for a dinucleotide are given below, which differ from one another in only one respect. In I the two mononucleotide groups are joined to one another through their carbohydrate groups, and the formula represents a substituted disaccharide. In II the two mononucleotide groups are joined to one another through their phosphoric acid groups and the formula represents a substituted diphosphoric acid.



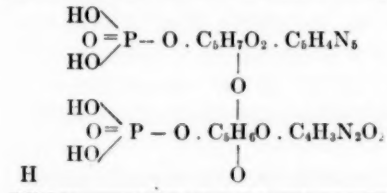
I



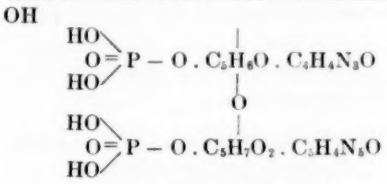
II

Formula I can properly be assigned to a dinucleotide that forms a *tetra*brucine salt, and is the most probable formula for the dinucleotide under discussion. Formula II cannot be assigned to a dinucleotide that forms a *tetra*brucine salt and therefore cannot represent the dinucleotide under discussion.

The mode of nucleotide linkage in the dinucleotide must of course exist also in yeast nucleic acid so far as two of its nucleotide groups are concerned, but it does not necessarily follow that this same mode of nucleotide linkage maintains throughout the entire nucleic acid molecule. However, in the following article we shall give evidence to show that such is the case, and that the relation between yeast nucleic acid and adenine-uracil dinucleotide is that which is expressed in the formula:



adenine-uracil dinucleotide.



yeast nucleic acid.

EXPERIMENTAL PART.

Hydrolysis of Yeast Nucleic Acid with Ammonia at 115°, and Separation of the Products.

A solution of 100 gm. of commercial yeast nucleic acid in 530 cc. of 2.5 per cent ammonia was heated for 1½ hours in an autoclave at 115°, and the cooled product was treated with 530 cc. of absolute alcohol. The bulky gelatinous precipitate thus formed was filtered on a Buchner funnel and drawn as completely as possible with a filter pump. The autoclave product is in this way separated into two fractions which may for convenience be designated as the guanine fraction and the adenine fraction. One fraction contains principally guanylic acid and forms an ammonium salt that is almost insoluble in 40 per cent alcohol. The other fraction is almost entirely adenine-uracil dinucleotide, whose ammonium salt is easily soluble in 60 per cent alcohol. When, therefore, a solution of the two fractions in ammonia is treated with an equal volume of alcohol, a sharp separation is effected.

The aqueous alcoholic solution of the dinucleotide was acidified with acetic acid, diluted with an equal volume of hot water, and treated with an aqueous solution of lead acetate as long as the reagent formed a precipitate with a portion of the cooled and filtered solution. About 450 cc. of 25 per cent lead acetate are required. When the precipitation was complete the solution was cooled and the heavy granular lead salt was filtered on a Buchner funnel and washed with a little cold water.

The lead salt was made into a thin paste with boiling water and decomposed with hydrogen sulfide. After the filtrate from lead sulfide had been carefully freed from every trace of hydrogen sulfide by boiling, it was evaporated at 50° under diminished pressure to about 50 cc., and treated with a large amount of absolute alcohol. The precipitated dinucleotide was hardened with absolute alcohol and dried with sulfuric acid in a vacuum desiccator.

From 300 gm. of yeast nucleic acid 174 gm. of the crude dinucleotide were obtained.

Adenine-Uracil Dinucleotide.

Specimens of the dinucleotide prepared in the manner described always contain a small amount of guanylic acid and by acid hydrol-

ysis produce a trace of guanine. The guanylic acid may be completely removed in the following way.

A solution of 100 gm. of the crude dinucleotide in 200 cc. of water is made alkaline with ammonia and treated with 300 cc. of absolute alcohol. The filtered solution is diluted with an equal volume of hot water, acidified with acetic acid, and precipitated with lead acetate as described, but the lead compound is washed with water, until every trace of soluble ammonium salt is removed. This can be done easily and without using the immense volume of wash water that would otherwise be necessary, by grinding up the lead precipitate to a thin paste with boiling water and drawing off the cooled liquid as completely as possible with a filter pump. After the washing in this way has been repeated two or three times, the wash water fails to give off ammonia with sodium carbonate in the cold.

The lead salt is decomposed with hydrogen sulfide, the filtrate from lead sulfide, after boiling until free from the gas, is evaporated, at 50° under diminished pressure, and the pale yellow syrup is treated with a large excess of alcohol. The precipitated dinucleotide is hardened and dried as described.

During the evaporation of the final aqueous solution a persistent bumping (due probably to the absence of ammonium salts) causes a serious loss of material so that the yield is lower than might have been expected. From 100 gm. of the crude dinucleotide 55 gm. of the purified product were obtained, or about 36 per cent of the commercial yeast nucleic acid used. The theoretical yield from pure dry nucleic acid is about 50 per cent. The dinucleotide is an amorphous white powder, soluble in water in all proportions, but insoluble in absolute alcohol. It responds to the color reactions for pentose with orcine and phloroglucine, and by acid hydrolysis yields an abundance of adenine but no trace of guanine.

A solution of 100 mg. of the substance in 2 cc. of 5 per cent sulfuric acid was heated for an hour at 100° and the hot solution was made alkaline with ammonia. No guanine was precipitated even after the solution had stood for a week, but upon the addition of ammoniacal silver nitrate, the fluid became solid with characteristic transparent gelatinous silver adenine. Tests for guanine and adenine made in this way never give misleading results.

The dinucleotide is levorotatory to polarized light. For determining the specific rotation, a preparation was used that had been dried in a vacuum over sulfuric acid and still contained 5 per cent of moisture. A solution of 3.3 gm. in 50 cc. of water gave a reading of $-0.85°$ when polarized in a 2 dm. tube. $[\alpha]_D = -6.8°$.

Preparation of Adenine from the Dinucleotide.—A solution of 5 gm. of dinucleotide in 100 cc. of 5 per cent sulfuric acid was heated for 1 hour at 100° when the product was made alkaline with ammonia and allowed to stand 24 hours. As no precipitation of guanine occurred, the solution was treated with silver nitrate in ammonia and the precipitated gelatinous adenine silver compound was suspended in water and decomposed with hydrogen sulfide. After filtering off the silver sulfide, the solution was evaporated to dryness on the water bath and the residue was crystallized first out of 5 per cent sulfuric acid, then out of hot water with the use of animal charcoal, and finally out of 1 per cent sulfuric acid. There were finally obtained 982 mg. of pure adenine sulfate. The united mother liquors obtained in the crystallization of adenine sulfate were made alkaline with ammonia and boiled until the excess of ammonia had been driven off. The solution was then decolorized with animal charcoal and treated with picric acid. There were finally obtained 189 mg. of adenine picrate. The total adenine obtained from 5 gm. of dinucleotide (5 per cent moisture) was 730 mg. instead of 950 mg.

Preparation of Adenosine from the Dinucleotide.[2]—The dinucleotide was dissolved in five parts of 2.5 per cent ammonia and heated in the autoclave for 2 hours at 135°. As no guanosine was deposited by cooling in ice water, the clear solution was treated with hot concentrated picric acid as long as cold picric acid still gave a precipitate, and the deposited adenosine picrate was recrystallized from hot water. From 15 gm. of dinucleotide we obtained 5.27 gm. of the picrate in glistening plates.

The picrate was dissolved in hot water and after the solution had cooled it was made and kept acid to Congo red with sulfuric acid as it was shaken out with ether for the removal of picric acid. The sulfuric acid was then neutralized with lead carbonate,[9] and

[9] This was to avoid the continual deposition of a barium compound during the subsequent evaporation, as is the case when barium carbonate is used.

the filtered solution was treated with hydrogen sulfide for the precipitation of a trace of lead. After evaporation of the filtrate from lead sulfide at 50° under diminished pressure and further concentration over sulfuric acid, the fluid was allowed to remain over night in the ice chest. A paste of crystalline adenosine was formed, from which pure adenosine was prepared in needle clusters, by recrystallization from hot water with the use of animal charcoal.

The substance was ash-free, contained no trace of free adenine, melted to a black liquid sharply at 219° (corrected), and contained one and one-half molecules of water of crystallization of which one molecule is driven off by heating in the air at 115°.[10]

0.3408 gm. air-dried substance lost 0.0210 gm. at 115°.

Calculated for $C_{10}H_{13}N_5O_4 \cdot \frac{1}{2} H_2O.H_2O$:	Found:
6.12	6.16

I. 0.1945 gm. dried at 115° required 13.35 cc. H_2SO_4 (1 cc. = 0.00370 gm. N).

II. 0.1233 gm. dried at 115° required 8.47 cc. H_2SO_4.

	Calculated for $C_{10}H_{13}N_5O_4 \cdot \frac{1}{2} H_2O$:	Found: I.	Found: II.
N	25.36	25.39	25.42

The picrate prepared from pure adenosine formed thin transparent plates with sharp edges, which decomposed and melted at 183.5° (corrected).

Preparation of Uracil from the Dinucleotide.—31 gm. of dinucleotide were heated in an autoclave at 140° for 3½ hours with 190 cc. of 25 per cent sulfuric acid. The product was diluted to 1 liter and precipitated with an excess of hot concentrated barium hydroxide. After filtration the excess of barium was removed by passage of carbon dioxide and the solution was evaporated under diminished pressure to about 250 cc. The concentrated fluid was acidified with nitric acid, the purine bases (adenine and hypoxanthine) were removed from the acid solution by the addition of silver nitrate, and the uracil was precipitated in the usual way with silver nitrate and barium hydroxide. The silver com-

[10] Levene and La Forge drove off one and one-half molecules of water from adenosine by heating at 110° in a vacuum over phosphorus pentoxide.

pound of uracil was suspended in hot water and decomposed with hydrogen sulfide. By evaporating the filtrate from silver sulfide to crystallization, there were obtained 5.18 gm. of crude uracil. This was recrystallized twice from 5 per cent sulfuric acid and finally from water with the use of animal charcoal. 2.618 gm. of pure uracil were obtained in macrocrystalline needles.

I. 0.2333 gm. required 15.78 cc. H_2SO_4 (1 cc. = 0.00370 gm. N).
II. 0.3262 " " 21.91 " "

	Calculated for $C_4H_4N_2O_2$:	Found: I.	II.
N	25.00	25.03	24.85

All fluids obtained in the purification of uracil were joined to the first mother liquor and examined for cytosine, but with negative results.

Preparation of Uridine from the Dinucleotide.[5]—A solution of dinucleotide in 2.5 per cent ammonia was heated in the autoclave for 2 hours at 133°. The product did not deposit guanosine when cooled in ice water for 2 hours, nor even upon standing over night in the ice chest. After warming to the room temperature the clear solution was treated with hot concentrated aqueous picric acid, as long as a drop of the fluid removed for the purpose was still found to give a precipitate with cold saturated picric acid. After completely precipitating the adenosine in this way, the solution was cooled and filtered. The excess of picric acid was removed with sulfuric acid and ether, the sulfuric acid was precipitated with hot concentrated barium hydroxide, and the solution was evaporated almost to dryness at 50° under diminished pressure. Alcohol was added, hydrochloric acid was passed in to the point of saturation, and the product was further treated as Levene and La Forge direct.[5]

Owing to the complication of the process we made no attempt to obtain a quantitative yield. From 100 gm. of air-dried dinucleotide there were obtained 7.3 gm. of snow-white crystalline uridine. (The yield from yeast nucleic acid is 5 per cent.)

I. 0.3883 gm. required 11.89 cc. H_2SO_4 (1 cc. = 0.00370 gm. N)
II. 0.3212 " " 10.02 " "

	Calculated for $C_9H_{12}N_2O_6$:	Found: I.	II.
N	11.47	11.33	11.54

The substance melted at 159° (corrected). Specimens of pure uridine from the dinucleotide and from yeast nucleic acid were heated in two capillary tubes on the same thermometer. The two specimens melted simultaneously at 159° (corrected).

Cytidine was not present in the first mother liquor obtained in the preparation of uridine. (When dealing with yeast nucleic acid, cytidine nitrate can always be prepared from this mother liquor.)

The Uracil Group in the Dinucleotide.—It has been suggested that the existence of a uracil group in nucleic acid has never been proven, and this is really the case if rigid proof be demanded. It is true that both uracil and uridine can be obtained from nucleic acid, but the corresponding amino compounds cytosine and cytidine are always found at the same time. The two oxy-compounds can easily be formed from the amino-compounds and might actually be produced in this way during the acid hydrolysis of nucleic acid, for neither of the oxy-compounds has been obtained from nucleic acid without employing hot mineral acid either in the hydrolysis itself or in the isolation of the product.

The failure to find any trace of cytosine among the hydrolytic products of the dinucleotide is evidence of considerable value but the question is finally decided by the chemical composition of the crystalline brucine salt of the dinucleotide.

Nitrogen required for the brucine salt of adenine-*cytosine* dinucleotide		10.05
Nitrogen required for the brucine salt of adenine-*uracil* dinucleotide		9.42
Nitrogen found.	I	9.35
	II	9.41
	III	9.35

A uracil group therefore exists in the dinucleotide and must also exist in the nucleic acid.

Partition of Phosphorus in the Dinucleotide.[8]—This subject is dealt with exhaustively in the contribution that follows,[11] where it is shown that half of the phosphoric acid comes off by mild hydrolysis with dilute mineral acid while half remains firmly bound. As far as this has evidential value it shows that the dinucleotide

[11] Jones, W., and Read, B. E., *J. Biol. Chem.*, 1917, xxix, 123.

is a mixed dinucleotide composed of groups of two mononucleotides, one a purine nucleotide and the other a pyrimidine nucleotide.

The Brucine Salt of the Dinucleotide.

A solution of 10 gm. of dinucleotide in 30 cc. of hot water was treated with a solution of 24.2 gm. of brucine in 48.4 cc. of hot alcohol. Crystals of the brucine salt appeared almost immediately. When the material had cooled to the room temperature, the crystals were filtered off, washed first with cold water, then with hot alcohol, and allowed to dry in the air. The salt weighed 21.12 gm. After recrystallization from fifty parts of hot water 10.4 gm. were obtained.

By repeated crystallization of the compound from hot water there is a considerable loss of material, due to its solubility in cold water. This can be recovered by careful evaporation of the mother liquors at a low temperature, but there is little need for recrystallization since the crystalline salt first obtained has the same melting point and elementary composition as the products of its recrystallization. The recrystallized salt was used in the analytical work reported in the next section.

When heated in a capillary tube the brucine salt contracts and recedes from the sides of the tube at 172–173°, but is not otherwise changed until the temperature reaches 174°. Between 174° and 175° the substance turns brown and melts. This same conduct is exhibited by all specimens of the brucine salt even after recrystallization from hot water five times. The hydrous salt contains fourteen molecules of water of crystallization and has the composition represented by the formula $C_{19}H_{25}N_7P_2O_{15}.4(C_{23}H_{26}N_2O_4).14H_2O$. The substance is very stable in the air at the room temperature, but slowly loses weight in a vacuum over sulfuric acid. Heated in the air at 110° it loses all of its water of crystallization and the weight of the anhydrous salt is not changed either by heating several hours longer at 125° or by heating at 110° in a vacuum over phosphorus pentoxide. The anhydrous salt takes up water and increases in weight when exposed to the air.

The material used for the following analyses was recrystallized from hot water once or oftener and except for the determinations of water of

crystallization was dried at 115°. The required values in the table below are for the formula $C_{19}H_{25}N_7P_2O_{15}.4(C_{23}H_{26}N_2O_4)$ except those for water of crystallization which are calculated from the formula $C_{19}H_{25}N_7P_2O_{15}.4(C_{23}H_{26}N_2O_4).14H_2O$. In determining the phosphorus, the substance was oxidized with potassium sulfate and concentrated sulfuric acid to which a few drops of 20 per cent copper sulfate were added.

For determining the brucine, the salt was suspended in fifty parts of hot water, and ammonia was added, until the substance passed into solution. Upon cooling, crystalline brucine was deposited, $C_{23}H_{26}N_2O_4.4H_2O$. This was filtered off and the filtrate was extracted with chloroform which was evaporated in a weighed platinum dish. The crystalline brucine was added and after drying at 110° the dish was again weighed. About 97 per cent of the brucine is precipitated by ammonia in crystalline form and is easily soluble in chloroform; about 3 per cent remains for the chloroform extraction.

I. 0.6219 gm. air-dried substance lost 0.0636 gm. at 115°.
II. 0.4947 " " " " 0.0502 " " 115°.
III. 0.2401 " dried at 115° gave 0.1269 gm. H_2O and 0.5265 gm. CO_2.
IV. 0.2561 " gave 0.1299 gm. H_2O and 0.5588 gm. CO_2.
V. 0.3596 " " 30.0 cc. N (25° and 768 mm.).
VI. 0.3920 " " 33.1 " N (26.5° " 760 ").
VII. 0.3418 " " 28.4 " N (25° " 766.5 ").
VIII. 1.3874 " " 0.1389 gm. $Mg_2P_2O_7$.
IX. 1.4243 " " 0.1419 " "
X. 1.2254 " " 0.2704 " $MgNH_4PO_4.6H_2O$.
XI. 0.4445 " " 0.3114 " brucine.
XII. 0.4756 " " 0.3344 " "

	H_2O	C	H	N	P	Brucine.
Calculated.	10.16	59.76	5.79	9.42	2.78	70.7
Found.						
I.....	10.23					
II.....	10.14					
III.....		59.80	5.87			
IV.....		59.51	5.64			
V.....				9.41		
VI.....				9.35		
VII.....				9.35		
VIII.....					2.79	
IX.....					2.77	
X.....					2.79	
XI.....						70.1
XII.....						70.3

Partition of Phosphorus in the Brucine Salt.[8]—Accurate determination of the partial phosphoric acid in the brucine salt is difficult because the brucine not only disturbs the hydrolysis but also interferes with the estimation of the products. The results therefore differ somewhat from the theoretical.

After first making a determination of total phosphoric acid, a specimen of the brucine salt was heated at 100° for 1 hour with twenty parts of 10 per cent sulfuric acid. The fluid, made alkaline with ammonia, was cooled, and filtered from precipitated brucine. The filtrate was treated with magnesia mixture and the ammonium magnesium phosphate weighed. From the amount of this substance obtained, the pyrimidine correction for 1 hour was subtracted.

1.2254 gm. gave 0.2704 gm. $MgNH_4PO_4.6H_2O$ (total).
0.9049 " " 0.1131 " " (partial).

The results are expressed in percentages of magnesium ammonium phosphate.

Total found	22.06
" required	22.00
Partial found	11.49
" required	11.00

THE MODE OF NUCLEOTIDE LINKAGE IN YEAST NUCLEIC ACID.

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(Received for publication, December 27, 1916.)

When a purine nucleotide is heated with dilute sulfuric acid its phosphoric acid is set free rapidly and completely. On the contrary, a pyrimidine nucleotide loses its phosphoric acid under the same conditions very slowly, so that the existence of purine and pyrimidine groups in mixed nucleotides can be indirectly ascertained from the rapidity with which their phosphoric acid is set free. Thus, yeast nucleic acid is a tetranucleotide composed of groups of two purine nucleotides and two pyrimidine nucleotides. As we should expect, half of its phosphoric acid is easily set free and half is firmly bound.

In the following are given the results obtained by the application of this method to adenine-uracil dinucleotide.

EXPERIMENTAL.

Eight portions of dinucleotide were heated with 5 per cent sulfuric acid (20 cc. per gm. of substance) for various periods of time from ½ hour to 7 hours, and the liberated phosphoric acid was determined as magnesium ammonium phosphate. In addition, the total phosphoric acid of the dinucleotide was determined after destroying the organic matter. The details of this procedure are described in former articles.[1] The results are given in Table I and for comparison the results formerly obtained with yeast nucleic acid are given in Table II.

These results are represented diagrammatically in Figs. 1 and 2. Hours are laid off as abscissæ and amounts of magnesium ammonium phosphate multiplied by 5 are laid off as ordinates. In Fig. 1 the upper curve is the locus of points that represent the

[1] Jones, W., *J. Biol. Chem.*, 1916, xxiv, p. iii; 1916, xxv, 87. Germann, H. C., *ibid.*, 1916, xxv, 189.

TABLE I.

Hydrolysis of Adenine-Uracil Dinucleotide.

Substance used.	Corrected for 3 per cent of moisture.	Time of heating.	Magnesium ammonium phosphate obtained.	Amount per gm. of dinucleotide.	Pyrimidine correction.	Amount from the purine nucleotide.
gm.	*gm.*	*hrs.*	*gm.*	*gm.*	*mg.*	*gm.*
0.6536	0.6340	½	0.1339	0.211	5	0.206
0.6450	0.6256	1	0.1790	0.286	10	0.276
0.8110	0.7867	2	0.2724	0.346	20	0.326
0.7738	0.7506	3	0.2704	0.360	30	0.330
0.8148	0.7904	4	0.2976	0.377	40	0.337
0.8378	0.8127	5	0.3102	0.382	50	0.332
0.8155	0.7910	6	0.3129	0.396	60	0.336
0.9627	0.9338	7	0.3750	0.402	70	0.332
0.6178	0.5993	Total.	0.4012	0.669	½ total =	0.335
0.5932	0.5754	"	0.3883	0.675		0.338

TABLE II.

Hydrolysis of Yeast Nucleic Acid.

Substance used.	Corrected for 8.5 per cent of moisture.	Time of heating.	Magnesium ammonium phosphate obtained.	Amount per gm. of nucleic acid.	Pyrimidine correction.	Amount from purine nucleotides
gm.	*gm.*	*hrs.*	*gm.*	*gm.*	*mg.*	*gm.*
0.7347	0.6723	½	0.1284	0.191	5	0.186
0.8644	0.7909	1	0.2315	0.292	10	0.282
0.9837	0.8991	2	0.3125	0.348	20	0.328
0.9251	0.8465	3	0.3038	0.359	30	0.329
1.0305	0.9429	4	0.3488	0.371	40	0.331
0.8333	0.7625	5	0.2876	0.377	50	0.327
0.9927	0.9083	6	0.3565	0.392	60	0.332
0.9667	0.8845	7½	0.3625	0.410	75	0.335
0.8179	0.7484	Total.	0.5112	0.644	½ total =	0.322

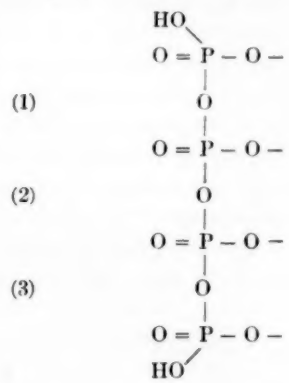
amount of magnesium ammonium phosphate actually obtained from the dinucleotide and it shows the rate at which phosphoric acid is liberated from both mononucleotide groups. The several points of the lower curve are obtained by calculation, *i.e.*, by subtraction of the pyrimidine correction, so that the curve shows the rate at which phospboric acid is liberated from the purine nucleotide group. In Fig. 2 yeast nucleic acid is similarly represented.

FIG. 1.

FIG. 2.

CONCLUSION.

The two figures are as nearly superimposable upon one another as could reasonably be expected. Therefore, so far as concerns its phosphoric acid linkages (but no farther), nucleic acid is composed of the groups of two dinucleotides identical with one another and identical with the nucleic acid itself. Hence the phosphoric acid linkage (2) in the diagram below does not exist.



Again, the two dinucleotides must have identical phosphoric acid linkages. If linkage (1) exists, linkage (3) must also; if linkage (1) does not exist, linkage (3) cannot. But it was shown in the preceding article that there is no direct phosphoric acid linkage in adenine-uracil dinucleotide and therefore none at the corresponding point in yeast nucleic acid. Hence both linkages (1) and (3) are excluded and there is no direct linkage of phosphoric acid groups in the entire nucleic acid molecule.

That the nucleotide groups of yeast nucleic acid (and adenine-uracil dinucleotide as well) are not united to one another through their phosphoric acid groups is proven. That this union is through the carbohydrate groups, we conclude from the general principles of physiological chemistry. The combination of purine groups with pyrimidine groups or of either with a carbohydrate group whose aldehyde affinity is already satisfied is very rare in physiological chemistry. But the polysaccharide structure is sufficiently common.

It is of course possible to draw other conclusions that can be adjusted to the facts, but they all involve the assumption of curious coincidences and compensations which would cause phosphoric acid to be liberated with equal ease from different kinds of linkage, or that the liberation of phosphoric acid from one kind of linkage is excessively slower than from another. After careful consideration of such matters, we believe we have drawn the correct conclusion.

THE INFLUENCE OF INTRAVENOUS INJECTION OF WITTE'S PEPTONE UPON THE SUGAR CONTENT OF THE BLOOD AND EPINEPHRINE HYPERGLYCEMIA AND GLYCOSURIA.

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(Received for publication, December 11, 1916.)

Henderson and Underhill reported that the intravenous injection of peptone into dogs causes hyperglycemia and glycosuria which they considered due to an accompanying acapnia. More recently McGuigan and Ross have stated that peptone administered to dogs intravenously induces a notable hypoglycemia and they ascribe the results obtained by Henderson and Underhill to anesthesia.

On the other hand, Glaessner and Pick claim that both pancreatic juice and peptone can inhibit epinephrine glycosuria. If Glaessner and Pick's report is compared with that of McGuigan and Ross, it is possible that the inhibitory influence of peptone upon epinephrine glycosuria may be due to the hypoglycemia caused by peptone. But it is also possible that peptone decreases the permeability of the kidney for sugar and thus inhibits epinephrine glycosuria, notwithstanding that the blood sugar may or may not be changed simultaneously. Though pancreatic extract has been shown by many investigators to have an inhibitory influence upon epinephrine glycosuria, Leschke demonstrated that an intravenous injection of pancreatic extract alone induces glycosuria. At the suggestion of Professor Frank P. Underhill, I have investigated the effect of peptone injection upon the sugar content of the blood and also upon epinephrine hyperglycemia and glycosuria.

Methods.

Full-grown rabbits were used. They were fed on oats and corn, greens being added to the diet from time to time. Water was

given freely. The urine was obtained by pressure on the bladder through the abdominal wall.

Witte's peptone was always employed in 10 per cent solution in 0.9 per cent sodium chloride solution. Unless otherwise noted, the peptone solution was always sterilized by boiling. Peptone (0.3 to 0.75 gm. per kilo of body weight) was injected into an ear vein without anesthesia. Blood samples for sugar determination were drawn from the other ear. For the control experiments an equal volume of 0.9 per cent sodium chloride solution was used. McGuigan and Ross assert that gelatin and other proteins can cause hypoglycemia as well as peptone. In accordance with this statement some experiments have been made to test the influence of gelatin and egg albumin upon blood sugar content. Commercial gelatin and a preparation of egg albumin were made up to 10 per cent solution with 0.9 per cent sodium chloride solution. The gelatin solution was sterilized by boiling, the egg albumin solution being used without sterilization.

When peptone and epinephrine were injected successively, peptone solution was first injected into an ear vein and immediately after that epinephrine (adrenalin chloride 1: 1000 Parke, Davis and Company) was administered subcutaneously.

The influence of intravenous injection of gelatin, egg albumin, and also soluble starch upon epinephrine hyperglycemia and glycosuria was studied in some experiments. The methods were the same as described with peptone and epinephrine. Soluble starch (Kahlbaum) was injected in 10 per cent solution, being dissolved in 0.9 per cent sodium chloride solution and sterilized by boiling.

In some cases body temperature, respiration, and pulse rate were determined. The body temperature was measured by inserting a thermometer into the rectum after collection of the blood samples. Respiration and pulse rate were measured directly before the taking of a blood sample.

The blood sugar was determined by the Lewis-Benedict method, as described by Miss McDanell in this laboratory. The sugar in the urine was estimated by a Schmidt and Haensch triple shadow saccharimeter after removal of the coloring matters and levorotatory substances by a saturated solution of mercuric acetate. Peptone, gelatin, and egg albumin added to the urine were shown not to interfere with this method. For the qualitative tests of sugar in the urine Benedict's reagent was employed.

The Influence of Intravenous Injection of Witte's Peptone upon the Sugar Content of the Blood.

When Henderson and Underhill injected Witte's peptone (0.3 to 0.63 gm. per kilo of body weight) intravenously into dogs, the sugar content of the blood increased as high as 0.26 to 0.27 per cent and the urine samples contained large amounts of sugar. McGuigan and Ross also injected Witte's peptone intravenously into dogs, using doses of about 0.12 to 0.66 gm. per kilo of body weight. In their experiments the sugar content of the blood reached a minimum about 2 hours after the peptone injection. The usual result was a fall of the sugar to about one-half or one-third of the original, which in general was much lower than that recorded by other investigators. In some cases they observed a transient and to them insignificant hyperglycemia in the interval between the injection of peptone and the hypoglycemic state; they ascribe this period of hyperglycemia to asphyxial conditions. Henderson and Underhill specifically called attention to the fact that the injection of Witte's peptone induces respiratory disturbances which they ascribed as being the cause of the hyperglycemia and glycosuria present. It is evident that McGuigan and Ross have confirmed the contention of Henderson and Underhill who made no statement concerning blood sugar content or glycosuria after peptone injection so arranged as to have no relation to respiratory disturbances. It is a well known fact that peptone is toxic, when administered intravenously (Underhill, Popielski). Underhill (1903) investigated its behavior with material of both animal and vegetable origin. The susceptibility to peptone is not the same in different species of animals. An intravenous administration of peptone usually brings forth severe toxic symptoms in dogs and sometimes kills the animals even in the dose used by Henderson and Underhill or McGuigan and Ross. The rabbit is usually considered to be less susceptible to peptone. Buchner and Geret reported that an intraperitoneal injection of pure peptone, specially prepared by themselves, in doses of 0.2 gm. per kilo of body weight, killed rabbits within 12 hours. In my own experiments the rabbits usually showed no severe symptoms except a slight degree of prostration after an intravenous injection of Witte's peptone in doses of 0.5 to 0.75 gm. per kilo of body weight. In a few cases 0.5 to 1.0 gm. of Witte's peptone per kilo

of body weight killed the rabbits immediately or in a short time. In such cases prostration, weak heart action, and convulsions were the most noticeable symptoms. The results of the experiments are shown in Table I.

TABLE I.

The Influence of Intravenous Injection of Witte's Peptone upon the Sugar Content of the Blood.

Rabbit.		Amount of peptone injected per kilo of body weight.	Blood sugar content (percentage).										
				Hrs. after injection.									
No.	Body weight.		Before injection.	¼	½	1	2	3	4	6	8	24	48
	kg.	*gm.*											
I	2.50	0.5	0.15	0.22		0.18	0.13	0.12	0.11	0.11		0.13	0.14
II	1.88	0.5	0.13		0.18	0.21	0.20	0.15	0.12	0.12	0.12	0.14	0.12
III	1.98	0.5	0.13	0.17	0.17	0.15	0.15	0.13	0.13	0.12	0.13	0.13	0.14
IV	1.84	0.5	0.14	0.15	0.19	0.23	0.20	0.18	0.14		0.11		
V	1.96	0.5	0.14		0.19	0.17	0.13	0.12	0.13				
VI	2.18	0.5	0.11			0.15	0.18	0.12	0.12				
VII	2.18	0.5	0.10			0.11	0.12	0.13	0.10				
VIII	2.80	0.75	0.11			0.18	0.16	0.12	0.10				
IX	1.90	0.75	0.12			0.16	0.16	0.12	0.10				
Average			**0.13**	**0.18**	**0.18**	**0.17**	**0.16**	**0.13**	**0.12**	**0.12**	**0.12**	**0.13**	**0.13**

Respiration (R), Pulse Rate (P), and Body Temperature (T).

Rabbit.		Before injection.	Hrs. after injection.								
			¼	½	1	2	3	4	6	8	24	48
	R.	88		92	90	88	92	120	100	120	98	80
II	P.	192		200	203	230	254	200	254	240	240	252
	T.	39.6		39.2	39.0	39.0	38.9	39.3	39.5	39.6	39.2	38.8
	R.	88		100	60	68	72	88	88	88	100	68
III	P.	140		148	200	192	164	192	168	168	180	160
	T.	38.1		38.3	38.8	38.4	38.6	39.1	39.3	38.5	38.8	38.5
	R.	68		76	80	80	68	80		76		
IV	P.	220		262	240	236	228	240		252		
	T.	38.5		38.4	38.6	38.7	38.9	39.2		39.4		
	R.	120		80	88	92	72	72				
V	P.	156		180	216	216	240	240				
	T.	38.3		38.1	38.4	38.6	39.3	39.0				

From the data in Table I, it is evident that *in the rabbit hypoglycemia is not induced by the intravenous injection of Witte's peptone, but, on the contrary, in most cases a slight hyperglycemia is invoked a few hours after the injection. Glycosuria does not develop.* Peptone injection had no remarkable influences upon the body temperature, respiration, and pulse rate. An excessive respiration, which was noticed by Henderson and Underhill in dogs, could not be demonstrated distinctly by rabbit experiments. The estimation of blood gases was not made in the present experiments. It cannot be stated here, therefore, whether the slight hyperglycemia, which was found in most cases of my experiments, is also due to acapnia or not.

To eliminate a possible error, which might be caused by the peptone itself circulating in the blood, a small amount of peptone was added to blood samples *in vitro*, and the sugar content of the mixture was estimated. The results showed that the method employed for the sugar determination was not interfered with by peptone itself.

Sodium chloride can cause glycosuria (salt glycosuria). But in order to obtain such results, a large amount of saline solution must be used. Underhill and Closson observed glycosuria after injecting about 90 cc. of $\frac{1}{2}$ molecular sodium chloride solution. In their experiments the glycosuria was accompanied by hypoglycemia. Bang could find neither glycosuria nor hyperglycemia after injecting about 100 cc. of 0.9 per cent NaCl solution (10 cc. in 10 minutes). It is a well known fact that emotion sometimes causes glycosuria. Cannon, Shohl, and Wright demonstrated that simply binding fast is enough to cause glycosuria in a cat. But nobody has observed such emotional glycosuria in rabbits. In Table II, two control experiments with 0.9 per cent sodium chloride solution instead of peptone solution are shown. The sugar content of the blood, respiration, pulse rate, and body temperature were not markedly affected.

McGuigan and Ross demonstrated that anesthesia can cause hyperglycemia. Epstein, Reiss, and Branower also reported that operations under anesthesia cause hyperglycemia in human beings; for example, before anesthesia the blood sugar content was 0.1 to 0.13 per cent, and after operation 0.17 to 0.27 per cent. In my experiments anesthetics were omitted. A hyperglycemia was also

TABLE II.

Control Experiments with the Saline Solution.

Rabbit.		Volume of 0.9 per cent NaCl solution injected.	Blood sugar content (percentage).					
			Before injection.	Hrs. after injection.				
No.	Body weight.			1	2	3	4	6
	kg.	*cc.*						
I	2.88	14.4	0.11	0.13	0.13	0.11	0.11	0.11
II*	2.80	21.0	0.13	0.11	0.12	0.12	0.11	0.11

Respiration (R), Pulse Rate (P), and Body Temperature (T).

Rabbit.		Before injection.	Hrs. after injection.				
			1	2	3	4	6
I	R.	80	76	88	100	104	96
	P.	240	252	276	240	264	264
	T.	38.7	39.1	39.2	39.4	39.0	38.5
II*	R.	84	80	88	88	92	104
	P.	216	252	240	240	240	264
	T.	38.8	38.6	38.7	38.6	38.6	39.3

* This was Rabbit VIII in Table I.

demonstrated after a considerable loss of blood (Bang). In my experiments the whole amount of blood taken for sugar estimation was not large enough for consideration of such a factor.

McGuigan and Ross thought that peptone is more active in causing a lowering of the blood sugar when it is dissolved in cold water and not boiled after solution. In order to find out if there is any such difference between unboiled and boiled peptone, unboiled peptone was injected into two rabbits. Here also, instead of lowering blood sugar, the level was more apt to increase (Table III).

After intravenous administration of peptone the sugar content of the blood sometimes increased as high as 0.2 per cent or more (Rabbits I. II, and IV in Table I). But in none of the cases was glycosuria observed. When Epstein and his coworkers found a slight hyperglycemia after operations under anesthesia they could not demonstrate any glycosuria. By testing the renal function with phenolsulfonephthalein in such cases, they found that

TABLE III.

The Influence of Intravenous Injection of Unboiled Peptone upon the Sugar Content of the Blood.

Rabbit.		Amount of peptone injected per kilo of body weight.	Blood sugar content (percentage).				
			Before injection.	Hrs. after injection.			
No.	Body weight.			1	2	3	4
	kg.	*gm.*					
I	2.14	0.5	0.10	0.12	0.12	0.17	0.10
II	1.86	0.5	0.12	0.15	0.15	0.14	0.14

the absence of glycosuria is due to a change of the renal permeability. In the case of peptone this explanation for the absence of glycosuria seems to be plausible, as a later description shows.

The Influence of Intravenous Injection of Gelatin and Egg Albumin upon the Sugar Content of the Blood.

McGuigan and Ross asserted that lowering of the blood sugar is the usual result of the intravenous injection of proteins, and this was proved by blood transfusion and the injection of gelatin. The results of my experiments in this direction are shown in Table IV.

TABLE IV.

The Influence of Intravenous Injection of Gelatin and Egg Albumin upon the Sugar Content of the Blood.

Rabbit.		Amount of substance injected per kilo of body weight.	Blood sugar content (percentage).				
			Before injection.	Hrs. after injection.			
No.	Body weight.			1	2	3	4
Gelatin.							
	kg.	*gm.*					
I	1.84	0.5	0.11	0.11	0.10	0.10	0.10
II	2.20	0.5	0.12	0.12	0.13	0.13	0.12
III	2.20	0.75	0.11	0.11	0.10	0.12	0.11
Egg albumin.							
IV	2.20	0.5	0.12	0.13	0.11	0.13	0.11
V	1.94	0.5	0.12	0.15	0.15	0.15	0.13
VI	1.94	0.75	0.11	0.10	0.09	0.09	0.09
VII	2.00	0.75	0.12	0.11	0.12	0.14	0.12

From these results it is apparent that *no noticeable influence of gelatin and egg albumin upon blood sugar content could be observed.* Neither glycosuria nor any severe disturbance of the general condition of the animals was observed.

The Influence of Intravenous Injection of Witte's Peptone upon Epinephrine Hyperglycemia and Glycosuria.

There are many substances which are said to inhibit epinephrine glycosuria. Zuelzer, and Biedl and Offer reported that they succeeded in inhibiting epinephrine glycosuria by injecting pancreas preparations. Biedl and Offer also obtained the same result by injecting the thoracic duct lymph. Glaessner and Pick inhibited epinephrine glycosuria by injecting pancreatic juice or Witte's peptone. Investigating the relations of the ductless glands, Eppinger, Falta, and Rudinger explained Zuelzer's experiments by saying that the excess sugar mobilized by epinephrine was oxidized by the pancreas extract injected simultaneously and therefore was not eliminated in the urine.

It is interesting to observe that Leschke induced glycosuria by injecting pancreatic extract, which is said to inhibit epinephrine glycosuria. Tomaszewski and Wilenko reported that sodium chloride solution, which acts as a lymphagogue, can inhibit epinephrine glycosuria. As to the influence of calcium chloride upon epinephrine glycosuria, the results of investigators are not concordant. Although Schrank demonstrated an inhibitory influence of this salt, Underhill (1916) was able to cause a noticeable increase of epinephrine glycosuria. In Underhill's experiments, after subcutaneous injection of calcium salt, epinephrine hyperglycemia was maintained, but its curve was distinctly modified. After confirming the results of Zuelzer, Biedl and Offer, and others, von Fürth and Schwarz succeeded in inhibiting epinephrine glycosuria by intraperitoneal injection of turpentine or aleuronat. They estimated the sugar content of the blood, nitrogen, sodium chloride, and sugar content of the urine, and reached the conclusion that the inhibitory influence of pancreas preparations, turpentine, or aleuronat upon epinephrine glycosuria is not specific, but is due to the damage of the renal function. They added that the damage of the kidneys cannot be judged by the amount of

urine alone, as in some cases the amount was not at all affected, while the glycosuria was markedly decreased.

So far as I am aware, the only investigation of the influence of peptone upon epinephrine glycosuria is that reported by Glaessner and Pick. According to their description, when a dog weighing 6.8 kg. received 5 cc. of epinephrine solution subcutaneously, 5 gm. of sugar were found in 425 cc. of the urine, but 4 days later when it received 2 gm. of Witte's peptone intravenously, and 5 cc. of epinephrine solution subcutaneously, no sugar was found in 400 cc. of the urine. The blood sugar was not estimated.

My experiments were made with rabbits and the results are detailed in Table V. As some rabbits could not tolerate 1 mg. of epinephrine and 0.5 gm. of peptone per kilo of body weight, the amount of peptone was reduced to 0.3 to 0.35 gm. per kilo of body weight.

It is to be noted from Table V that *the grade of hyperglycemia produced by epinephrine was not markedly changed by an intrave-*

TABLE V.

The Influence of Intravenous Injection of Witte's Peptone upon Epinephrine Hyperglycemia and Glycosuria.

Rabbit.		Amount of substance injected per kilo of body weight.		Blood sugar content (percentage).						Urine (in 7½ hrs.).		
				Before injection.	Hrs. after injection.							
No.	Body weight.	Epinephrine.	Peptone.		1½	3	4½	6	7½	Volume.	Sugar.	
	kg.	*mg.*	*gm.*							*cc.*	*per cent*	*gm.*
IA*	2.06	1.0		0.10	0.31	0.32	0.31	0.28	0.15	128	**3.31**	**4.24**
IB	2.08	1.0	0.3	0.11	0.27	0.30	0.29	0.27	0.19	108	0.48	0.51
IC	2.04	1.0				0.32	0.34			100	**3.20**	**3.20**
II	1.84	1.0		0.13	0.35	0.32	0.30	0.25	0.20	140	**2.50**	**3.53**
III	1.64	1.0	0.5	0.12	0.32	0.30	0.13	0.10	0.09	144	0.19	0.27
IV	1.92	1.0	0.5	0.11	0.26	0.24	0.32	0.36	0.32	49	0.61	0.30
VA**	2.16	1.0	0.35	0.11	0.28	0.30	0.33	0.31	0.26	60	0.21	0.13
VB	2.10	1.0				0.36	0.33			53	**3.94**	**2.09**

* Three experiments, IA, IB, and IC, were carried through with Rabbit I at intervals of 1 week.

** Two experiments, VA and VB, were carried through with Rabbit V with an interval of 1 week.

nous injection of peptone. But epinephrine glycosuria was distinctly decreased by peptone. Averaging the amounts of sugar in the urine, *3.3 gm.* of sugar were eliminated after the injection of epinephrine alone, while the amount of sugar was only *0.3 gm.* when peptone was injected simultaneously. It is a well known fact that the same dose of epinephrine may not produce the same degree of glycosuria in two different animals or in the same animal at different periods. But this seems to be insufficient to explain the data described above. The explanation of the decrease of epinephrine glycosuria, suggested by von Fürth and Schwarz, when pancreas preparations, turpentine, or aleuronat were injected simultaneously, *i.e.*, the damage of the renal function, may be applied here. Their statement that the renal permeability for sugar cannot be judged by the amount of urine only is also here confirmed. The absence of glycosuria, where a significant hyperglycemia was caused by administration of peptone alone (Table I), may also be explained in the same manner.

The Influence of Intravenous Injection of Gelatin, Egg Albumin, and Soluble Starch upon Epinephrine Hyperglycemia and Glycosuria.

When gelatin or egg albumin was injected intravenously just before epinephrine (1 mg. per kilo of body weight) injection, the amount of both gelatin and egg albumin had to be reduced so as to be less than 0.2 gm. per kilo of body weight. All rabbits in which gelatin or egg albumin was injected with a dose over 0.2 gm. per kilo of body weight died immediately or within a few hours. The heart action was markedly affected. It is conceivable that the colloidal nature of the protein injected may be responsible for the death of the animals after epinephrine introduction. To determine this point experiments have been carried through with a colloidal solution of a different nature, soluble starch. When soluble starch was employed in place of gelatin or egg albumin, the general condition of the animal was not changed. The results are shown in Table VI.

It is to be noted from Table VI that *the grade of hyperglycemia called forth by epinephrine was not markedly changed by the intravenous injection of gelatin or egg albumin; but epinephrine gly-*

TABLE VI.

The Influence of Intravenous Injection of Gelatin, Egg Albumin, and Soluble Starch upon Epinephrine Hyperglycemia and Glycosuria.

Rabbit.		Amount of substance injected per kilo of body weight.		Blood sugar content (percentage).		Urine (in 7½ hrs.).		
No.	Body weight.	Epinephrine.	Gelatin, egg albumin, or soluble starch.	1½ hrs. after injection.	3 hrs. after injection.	Volume.	Sugar.	
Gelatin.								
	kg.	*mg.*	*gm.*			*cc.*	*per cent*	*gm.*
I	1.66	0.75	0.2		0.21	37	0	0
II	1.46	1.0	0.15	0.31	0.34	59	0.77	0.45
III	2.24	1.0	0.15	0.31	0.32	131	0.85	1.11
Egg albumin.								
IV	1.68	1.0	0.15	0.29	0.37	35	1.03	0.36
V	1.70	1.0	0.075	0.28	0.31	109	0.63	0.69
Soluble starch.								
VI	2.54		0.5	0.11	0.10	109	0	0
VII	2.36	1.0	0.25	0.41	0.39	104	1.51	1.57
VIII	2.28	1.0	0.5	0.37	0.37	86	3.53	3.04

cosuria was distinctly decreased by them. Soluble starch seems to have no marked influence upon either hyperglycemia or glycosuria.

When soluble starch was injected without epinephrine (Rabbit VI in Table VI), the sugar content of the blood showed a normal value. The urine, collected 7½ hours after the injection, contained no reducing sugar, but showed a strong dextrorotation. When Mendel and Mitchell injected soluble starch intraperitoneally into a dog and a rabbit, they found a dextrorotatory substance in the urine, and considered this substance as the injected carbohydrate altered only slightly, if at all. Owing to the presence of such a dextrorotatory substance, which cannot be removed by mercuric acetate, the urinary sugar of the starch experiments was estimated by Pflüger's gravimetric method.

Ellinger and Seelig demonstrated that severe bacterial infection

and cantharidine nephritis markedly diminish epinephrine glycosuria in rabbits. Under these circumstances the function of the kidneys was severely affected, the urine containing a large amount of blood or protein, and the volume of the urine being distinctly diminished.

Injecting Witte's peptone and egg white subcutaneously into rabbits, de Waele and Vandevelde studied the fate of the injected substances. Witte's peptone was injected in doses of 0.2 to 1.0 gm., egg white in doses of 10 to 25 cc. The function of the kidneys was not markedly impaired. On the whole, the difference between the urine volume and nitrogen elimination, before and after the injection, was very slight. When peptone was injected, traces of albumin or peptone were sometimes found in the urine. After the injection of egg white, a small amount of protein and also sometimes a trace of peptone were demonstrated in the urine. Though the test of the renal function by nitrogen or chloride estimation was not made in my present work, it is obvious that the renal permeability for sugar is distinctly decreased by intravenous injection of gelatin and egg albumin, notwithstanding that the urinary flow was not always affected. It is interesting to note that soluble starch, which has a colloidal character as well as peptone, gelatin, and egg albumin, behaves differently in this respect.

SUMMARY.

The blood sugar content of rabbits shows a tendency to increase after intravenous injection of Witte's peptone in doses of 0.5 to 0.75 gm. per kilo of body weight.

When hyperglycemia obtains, it continues for a few hours only.

In no case was there any evidence of a hypoglycemia alleged by McGuigan and Ross to be induced in dogs by peptone injection.

In the present investigation no difference could be observed upon the blood sugar content between boiled and unboiled peptone solution.

The intravenous injection of gelatin or egg albumin has little or no influence upon the sugar content of the blood.

Although an intravenous injection of peptone may be without marked influence upon epinephrine hyperglycemia, glycosuria

is distinctly diminished. The influence of peptone is ascribed to a possible change in renal permeability for sugar.

The influence of intravenous injection of gelatin and egg albumin upon epinephrine hyperglycemia and glycosuria is apparently the same as that described for peptone.

The fatal results experienced with a combination of protein and epinephrine injections cannot be ascribed to the colloidal character of the proteins, since soluble starch is without any detrimental influence under similar experimental conditions. Soluble starch exerts no action upon blood sugar content of normal rabbits or upon epinephrine hyperglycemia and glycosuria.

I desire to express my thanks to Professor Frank P. Underhill for his suggestions, help, and criticism, and also to Professor Lafayette B. Mendel for his advice.

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VARIATION IN THE AMOUNT OF PHOSPHATIDES IN THE CORPUS LUTEUM OF THE SOW DURING PREGNANCY.*

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Morphological studies of the corpus luteum have led several investigators to the view that the lutein cells contain considerable amounts of lipoids. Chauffard, Laroche, and Grigaut,[1] for instance, have laid emphasis upon the cholesterol content of the organ, Cesa-Bianchi[2] and others upon the lecithin or upon lipoid bodies of a less definitely indicated nature. A direct chemical confirmation of these results has been given by the recent analyses of Fenger[3] who finds that the corpora lutea of pregnant cows contain about thirteen times as great an amount of petroleum ether-soluble phosphorus as lean muscle, which he took as a standard of comparison.

The author has been investigating the microchemical reactions of the lutein cells of swine, and has also concluded that these cells contain an unusual amount of a lipoid or mixture of lipoids of the phosphatide group. In this species, the treatment

* An account of the cytological side of the work will appear in the *Anatomical Record* at an early date. The writer wishes to express his thanks to Professor T. Brailsford Robertson for advice on matters of biochemistry, and for the privileges of his laboratory; and to Mr. Ralston B. Brown, Superintendent of the Oakland Meat Company, for provision of fresh tissues.

[1] Chauffard, A., Laroche, G., and Grigaut, A., Fonction cholestérinigénique du corps jaune, *Compt. rend. Soc. biol.*, 1912, lxxii, 223, 265.

[2] Cesa-Bianchi, D., Di alcune particolarità di strutturi e dei fenomeni di secrezione del corpo luteo, *Internat. Monatschr. Anat. u. Physiol.*, 1908, xxv, 1.

[3] Fenger, F., Phosphatides in the ductless glands, *J. Biol. Chem.*, 1916, xxvii, 303.

of the corpus luteum with slow aqueous fixing fluids causes the rounding up into droplets of a substance which during the life of the tissue is diffusely present in the periphery of the cells. The droplets thus caused to appear are soluble in alcohol of 60 per cent or stronger, in ether, chloroform, acetone, benzene, and xylene; they stain blue with Nile blue sulfate, pale brick red with neutral red, yellowish with osmium tetroxide (changing to gray in alcohol), are positive with Ciaccio's method, and give a brown lake with Weigert's hematoxylin; they are not anisotropic.

Alcoholic extracts of the corpora lutea of swine contain, mixed with neutral fat, a fat-like substance of pasty consistence which is yellowish in color, readily becoming brown in the air, and presenting a characteristic "greasy" odor. It gives microchemical reactions exactly like those mentioned above for the droplets in the cells, except that as it rounds into spheres when in contact with water, the spheres are momentarily anisotropic.

The tests cited indicate that the substance, thus revealed by a fortunate artifact of fixation, is probably a lipoid or lipoid mixture of the phosphatide series, containing perhaps a fatty acid (suggested by the partial reduction of osmium tetroxide). Although Fenger has shown the presence of large amounts of phosphatides in the corpora lutea of cows, the microscopical appearances described above cannot be found in this species. Perhaps the lipoids are here in a less oily condition, not rounding up in water so readily; or they are dissolved in other cell lipoids which themselves do not produce the peculiar spheres which have been mentioned. In the dog the appearances are exactly as in swine, and certain bodies seen by Cohn[4] in the lutein cells of rabbits are almost certainly due to the same cause.

But the point of greatest interest is that the lipoid made visible by this method is present only during the earlier part of pregnancy, and cannot be detected after about the middle of the term. Chemical estimations of the alcohol-soluble phosphorus in the corpora lutea of swine also show a decrease in the amounts of the phosphatides in the advancing stages of pregnancy. In order to determine this point, the ovaries of sows at desired stages were removed immediately after slaughtering, and the corpora lutea

[4] Cohn, F., Zur Histologie und Histogenese des Corpus luteum und des interstitiellen Ovarialgewebes, *Arch. mikr. Anat.*, 1903, lxii, 745.

were shelled out of their capsules. The substance was desiccated by grinding in a mortar with three times their quantity by weight of a mixture of equal parts of anhydrous sodium and calcium sulfates, and drying the resultant paste to a powder over a water bath (Leathes[5]). The coarse powder was more finely pulverized and extracted with absolute alcohol in the Soxhlet apparatus for 3 days. Portions of the extract representing 5 gm. of the fresh gland were examined for phosphorus by von Wendt's modification of Neumann's method.[6] In the following table the phosphorus is expressed as P_2O_5, the figures therefore giving a relative indication of the amount of alcohol-soluble phosphorus-containing substances at the given stages.

	P_2O_5 per gm. of fresh tissue. *mg.*
Embryos 7 to 13 mm. long (early pregnancy)	0.63
Fetuses 98 to 140 mm. long (middle pregnancy)	0.43
Fetuses 190 to 270 mm. long (late pregnancy)	0.39

It cannot be too strongly emphasized that the corpus luteum is a very rapidly changing tissue. From the day of its sudden appearance until its atrophy, perhaps months later, its morphological appearance is in constant flux, and chemical studies must in the end be made with corpora lutea collected at known stages of the reproductive cycle, if they are to throw any light upon the physiology of the gland. The question naturally arises as to the rôle of the lipoids of the corpus. Robertson[7] has shown that the bodies of young animals contain a much higher percentage of alcohol-soluble phosphorus than those of the old. The corpus luteum of early pregnancy is, in effect, an islet of juvenile tissue among the older cells of its host. Is the larger amount of phospholipins, at this stage, merely an accompaniment of the youthfulness of the organ, or is it, as Fenger suggests, connected with the special secretory activities of the cells? The question offers possibilities of experimental test.

[5] Leathes, J. B., The Fats, London, 1910.

[6] Von Wendt, G., Untersuchung über den Eiweiss- und Salz-Stoffwechsel beim Menschen, *Skandin. Arch. Physiol.*, 1905, xvii, 211.

[7] Robertson, T. B., Experimental studies on growth. II. The normal growth of the white mouse, *J. Biol. Chem.*, 1916, xxiv, 380.